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MANURIAL STUDIES ON THE GRASSLANDS OF BOMBAY

I. EFFECTS OF MANURING ON THE CHEMICAL COMPOSITION *HEYLANDIA LATEBROSA* DC.

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Received: December 20, 1960

In a previous communication (Shankar and Bharucha, 1961) *Heylandia latebrosa* DC. (*godhadi*) was noted as a common legume of promising nutritional qualities, capable of establishing itself well, and thriving luxuriantly in the hilly grasslands of Raita, near Bombay, when protected from overgrazing. Burns *et al*. (1916) had reported that it could form a thick carpet in grasslands. Whyte (1957) stated that this legume gave favourable response in yield trials conducted over two seasons at Dharwar in Mysore State. By the middle of October, *Heylandia* will have dried up at Raita, and its proportion in the herbage will be very small since the grasses, whose grand period of growth lies between September and October, arrest its growth by overshading it. In September, however, it is luxuriant and forms a substantial part of the herbage.

In a manurial trial (randomized block experiment) conducted on the hilly grasslands of Raita, it was noticed that in the experimental plots, *Heylandia* was dominant in association with tall grasses like *Themeda triandra*, *Pseudanthistiria heteroclita* and *Ischaemum aristatum*. It was well relished by cattle, particularly the buffaloes. The experimental technique consisted in having three blocks of eight plots, each of 5½ sq. yards. The fertilizer treatments consisted of individual application of ammonium sulphate, superphosphate and calcium carbonate as well as their various combinations. The quantities of manures applied per acre were as follows: ammonium sulphate, 100 lb.; superphosphate, 200 lb.; and calcium carbonate, 100 lb.

In these days of intensive search for promising indigenous legumes and their establishment in grasslands for improving the nutritive qualities of herbage, it was felt necessary to determine the effects of manuring on the chemical composition of this important indigenous legume and compare it with that of a well-known exotic legume of proved merit. In the present investigation, separate yield trials of the legume could not be conducted since, in the natural state, it occurs mixed closely with grasses. However, this legume was found to yield 6,000 lb. to 7,000 lb. of herbage per acre.

The chemical composition of this legume, as affected by manuring with ammonium sulphate, superphosphate and calcium carbonate, are reported in this paper. A chemical analysis of the legume without manuring has been reported earlier (Shankar and Bharucha, *loc. cit.*).

METHODS

Plant material was collected in the third week of September at the best growth period of the legume and analyzed for protein-nitrogen by the micro-Kjeldahl method,

and for minerals by methods suggested by A.O.A.C. (1950).

RESULTS

Tables I and II compare the chemical composition of *Heylandia* with that of *berseem* (cf. Whyte, *loc. cit.*).

TABLE I. CRUDE PROTEIN (PERCENTAGE ON OVEN-DRY BASIS)

Treatment	*Heylandia*	*Berseem* (Whyte, 1957)
Control	8·968	20·500
N	17·500	14·030
P	18·836	18·800
Ca	16·681	19·381
Ca + P	17·330	21·360
P + N	14·230	23·550
N + Ca	14·225	23·480
N + P + Ca	18·080	21·650

TABLE II. MINERALS (PERCENTAGE ON OVEN-DRY BASIS)

Treatment	*Heylandia*			*Berseem* (Whyte, 1957)		
	Ash	CaO	P_2O_5	Ash	CaO	P_2O_5
Control	7·219	1·010	0·138	12·210	2·650	1·140
N	6·504	1·073	0·211	14·030	2·560	1·160
P	7·481	1·270	0·180	11·990	2·720	1·180
Ca	6·777	1·197	0·200	13·250	2·780	1·220
Ca + P	5·979	1·016	0·202	13·230	2·600	1·190
P + N	6·995	1·343	0·243	13·030	2·760	1·190
N + Ca	6·995	1·106	0·139	12·980	2·630	1·140
N + P + Ca	6·996	1·065	0·201	11·950	2·620	1·120

The results indicate that with regard to crude protein, *Heylandia* responds to manuring very well as compared to *berseem*, where the protein content was already high due to good nodulation (Whyte, *loc. cit.*). The maximum increment in crude protein content effected by superphosphate shows the need of phosphate application

to this grassland. The individual application of P, N or Ca has produced better effects than the mixtures containing any two of these fertilizers.

Because of the poor mineral status of these soils (Bharucha and Shankarnarayan, 1958), the mineral contents of this legume are generally low in comparison with that of *berseem*. The ash and calcium contents of *Heylandia* from the manured plots are not much different from those of untreated plots. Such feeble response of herbage calcium has been noticed by earlier Indian workers as well (Dabadghas, 1954). This legume, however, is very poor in the phosphorus content as compared to *berseem*, but manuring with N, P, or Ca improves the percentage of this nutrient to a certain extent.

Comparison of the chemical composition of this species with *berseem* reveals that after manuring, its crude protein content compares favourably with that of manured *berseem*. Its calcium content is quite good, considering that it is an indigenous legume growing in a hilly grassland of rocky nature such as Raita. The P_2O_5 content, however, is very low even after manuring, and requires further improvement. However, *Heylandia* deserves to be considered for establishment in soils of rocky nature, since it can establish itself well when protected from grazing and is quite hardy.

SUMMARY

The present investigations report the changes effected in the chemical composition of *Heylandia latebrosa* DC., a common legume of Raita grasslands near Bombay, which is well relished by cattle, particularly the buffaloes. Individual application of ammonium sulphate, superphosphate or calcium carbonate at the rate of 100 lb., 200 lb. and 100 lb. per acre is found to have improved the protein content of this species to a favourable degree comparable to that of *berseem*, a legume of established merit and feeding qualities. CaO and P_2O_5 contents are generally poorer than in *berseem* though P_2O_5 is improved by manuring to some extent. Maximum response in crude protein content is obtained when superphosphates are applied, implying the necessity of phosphate fertilization to these grasslands.

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FACTORS AFFECTING THE VIABILITY OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY*

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Received: January 25, 1961

Among pathogens causing destructive diseases of economic plants, *Sclerotinia sclerotiorum* (Lib.) de Bary occupies a rank of major importance, not only because of its phenomenally wide host-range, but also because of its ubiquitous distribution. Under the humid conditions obtaining in the submontane districts of the Punjab State, this fungus is the cause of a destructive stem-rot of gram (*Cicer arietinum* L.) and *berseem* or Egyptian clover, both of which are crops of great economic importance.

Sclerotia constitute one of the most important links in the life-history of this pathogen which persists from year to year in the form of these black and hard bodies (Ramsey, 1925; Brooks, 1928; Smith, 1931; Mundkur, 1934; Blackford, 1944; Staehelin, 1945; Moore *et al.*, 1949; Baker and Davis, 1951). An investigation of the factors affecting the viability of sclerotia, therefore, is considered to be basic to an effective programme of measures to combat the various diseases caused by this pathogen.

Brown and Butler (1936) state that sclerotia remain viable for 11 years under dry conditions in the laboratory. Sclerotia, 4½ years old, were found to be viable by Baker and Davis (*loc. cit.*). According to Ramsey (*loc. cit.*), various investigators have found that dry sclerotia remain alive for several years, but the consensus of opinion is that most of them decay in nature during the first year. Joshi (1924) also states that sclerotia do not seem to remain viable for very long periods—only 14 per cent germinating one year after development, whether in the field or in culture. This statement receives further support from Davis (1925), who also believes that under natural conditions, sclerotia do not remain viable, at least near the surface of the soil, for longer than one year.

Survival of sclerotia of *S. sclerotiorum* mixed with seeds of *Centaurea cyanus* has been recorded by Baker and Davis (*loc. cit.*). Sclerotia of this fungus, mixed with seeds of sunflower, pea, bean, red clover, white clover, crimson clover, radish, carrot, pepper, dahlia, soyabean, Jerusalem artichoke, alfalfa and some other plants, have been reported by Bisby (1924), Young and Morris (1927), Alcock (1931), Crosier (1936), Doyer (1938) Moore (1946) and Baker (1947). The writer has observed sclerotia mixed with the seed of gram (*Cicer arietinum* L.) obtained from diseased fields at Gurdaspur in the Punjab State.

According to Joshi (*loc. cit.*), sclerotia were killed by immersion for five minutes in hot water at 50°C, but they seemed to be very resistant to dry heat. Mundkur (*loc. cit.*) reported that they were viable after an exposure of four hours at 60°C in a hot-air oven. They, however, failed to germinate after being heated for one hour at

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*Part of the thesis submitted for Ph.D. degree of the University of Minnesota

50°—55°C, as recorded by Grooshevoy *et al.* (1941), who conclude that temperatures developing in compost heaps can kill them.

Death of sclerotia as a result of invasion by a *Trichoderma* has been recorded by Taubenhaus and Ezekiel (1932). According to Hino and Endo (1940), *Trichoderma viride* is parasitic on them and, according to Campbell (1947), *Coniothyrium minitans* is not only parasitic but also destroys them.

Brooks (1942) showed that a combination of flooding and soil treatment with cyanamide would kill a large percentage of sclerotia in many soils. According to Moore (1949), sclerotia decayed completely within 23 to 45 days in flooded marl, muck or sandy soils.

According to Walker (1950), sclerotia are admirably suited to over-wintering under snow in cold countries.

Beach (1923) tried various chemicals including copper sulphate, sulphur, corrosive sublimate, wood-alcohol and crude cresol in quantities non-toxic to lettuce, but none of them killed all the sclerotia of this pathogen.

This paper deals with the effect of some chemical, physical and biotic factors on the viability of sclerotia of the Punjab race of *S. sclerotiorum.*

EXPERIMENTAL

Chemical Factors

Effect of various chemicals: The effect of disinfectants or fungicides on the viability of air-dried sclerotia produced in culture is given in Table I. The treated sclerotia were washed in three changes of sterilized water to remove the chemicals and the untreated (control) sclerotia were surface-sterilized with 0·1 per cent mercuric chloride solution for five minutes and similarly washed with sterilized water thereafter. For tests of viability they were incubated at laboratory temperature (22°—25°C) on Richards's solution agar. Twenty-five sclerotia were used for each treatment.

TABLE I. EFFECT OF DIFFERENT CHEMICALS ON THE VIABILITY OF SCLEROTIA

Chemicals	Percentage of viable sclerotia after exposure for				
	5	10	15	30	60
	(..................		minutes		..................)
0·1 per cent mercuric chloride solution	100	100	100	100	0
1 per cent formalin solution	100	100	100	100	0
1 per cent sodium hypochlorite solution	100	100	100	100	100
1 per cent copper sulphate solution	100	100	100	100	100
Concentrated sulphuric acid	100	100	0	0	0
Absolute alcohol	100	100.	100	100	0
Sclerotia receiving no chemical treatment (control)	Percentage viability: 100				

These results show that sclerotia are highly resistant to the action of chemicals. They are not killed even by an exposure of one hour to one per cent solution of copper sulphate and sodium hypochlorite. They can endure the effect of 0·1 per cent solution of mercuric chloride and one per cent solution of formalin for 30 minutes without any reduction in their viability. They can even withstand absolute alcohol for a similar period and their survival after a ten-minute soak in concentrated sulphuric acid is very remarkable. Their resistance to chemicals is evidently due to their hard, black protective rind.

Physical Factors

(1) *Effect of moist heat:* Air-dried sclerotia were placed in small cheese-cloth bags and dipped for periods varying from 1 to 30 minutes in hot-water baths maintained at 50°, 60° and 70°C. The results are presented in Table II.

TABLE II. EFFECT OF HOT WATER OF DIFFERENT TEMPERATURES ON THE VIABILITY OF SCLEROTIA

Temperature of hot water in °C	*Percentage of viable sclerotia after exposure for						
	1	5	10	15	20	25	30
	(.........			minutes			.........)
50	100	0	0	0	0	0	0
60	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0
Sclerotia not receiving hot-water treatment (control)	Percentage viability: 100						

*Based on 25 sclerotia at each temperature.

Evidently, sclerotia are extremely susceptible to the lethal action of hot water. They cannot survive even an exposure thereto for five minutes at 50°C, thus corroborating the observation of Joshi (*loc. cit.*). Even an exposure for only one minute to 60° or 70°C is sufficient to kill them.

Hot water is promising as a means of killing sclerotia mixed with the seeds of various crops from which it is generally difficult to separate.

(2) *Effect of dry heat:* Air-dried sclerotia were put into small glass test-tubes and exposed for periods varying from half an hour to six hours to dry heat in hot-air ovens maintained at constant temperatures of 60°, 70°, and 80°C. Twenty-five sclerotia were used in each test. The results are given in Table III.

Exposure to dry heat at 60° or 70°C for six hours did not kill the sclerotia. Even at 80°C, 32 per cent of them survived. Mundkur (*loc. cit.*), however, had observed that exposures to dry heat of 60°C for periods above four hours were completely lethal. In tests made by Grooshevoy *et al.* (*loc. cit.*), sclerotia did not even survive an

exposure of one hour to a temperature range of 50°—55°C. Although these differences in results cannot be accounted for, it is possible that these investigators may have been dealing with different races of this pathogen. The amount of initial moisture in the sclerotia may also be the cause of variation in the results. It may be pointed out that in the present investigations the sclerotia were air-dried at room temperature for four days.

TABLE III. EFFECT OF DRY HEAT ON THE VIABILITY OF SCLEROTIA

Temperature in °C	Percentage viability of sclerotia after exposure for						
	½	1	2	3	4	5	6
	(............hours............)						
60	100	100	100	100	100	100	100
70	100	100	100	100	100	100	100
80	100	100	100	100	100	100	32
Sclerotia not exposed to dry heat (control)	Percentage viability: 100						

When recording the results of this experiment, an interesting observation has been made. Whereas on agar, where the control (unheated) sclerotia were as yet growing slowly and the colonies resulting therefrom had not produced any new crop of sclerotia, those which had been exposed to 60°C for half an hour to six hours had formed vigorous, fluffy cultures with large, fully formed sclerotia. This interesting point could not be pursued further, but there is a definite indication that dry heat at a temperature like 60°C, instead of damaging the viability of sclerotia, actually seems to stimulate them.

(3) *Effect of continuous freezing, and of freezing and thawing:* Sclerotia used in this experiment were divided into four lots. One lot was kept at room temperature without any treatment to serve as control. The remaining three lots were put separately into 150-ml. Erlenmayer flasks, each containing 50 ml. of sterilized distilled water. One of these three lots was frozen continuously for 14 days at 15°C in a deep-freeze cabinet. The second lot was frozen at the same temperature but was thawed at room temperature (22°—25°C) on alternate days. This treatment of alternate freezing and thawing, each of 24 hours' duration, was also continued for 14 days. The third lot, immersed in water, was kept at room temperature throughout this fortnight. The viability of the treated lots and of the control series was tested on agar and was found to be unimpaired.

Thus, the sclerotia of this fungus can withstand prolonged freezing at a very low temperature without loss of viability. Repeated fluctuations of temperature from 15° to about 25°C over a fortnight were also harmless to them. Likewise, a fortnight's immersion in water did not reduce their viability.

(4) *Effect of prolonged exposure to snow:* Glass tubes, open at both ends, were fixed in a 12-inch flower-pot filled with moist soil. About 4-month-old sclerotia, produced in culture, kept in air-dried condition at room temperature and showing 100 per cent viability, were placed at depths of 12, 9, 6 and 3 inches in the tubes and were covered with moist soil. Sclerotia were also placed in the open on November 16, 1950 and they became covered with snow immediately and remained so for four months, when the experiment was terminated. During this period, the temperature fell several times below 0°F, and occasionally touched the −25°F mark. Sclerotia were thus exposed to a prolonged and severe natural refrigeration. The frozen pot with the sclerotia was thawed at room temperature and the viability of the sclerotia was tested immediately. Sclerotia were found to be viable to the extent of 100 per cent, regardless of the depth of burial in the pot. The results of this experiment not only corroborate the findings of other workers, but also show that the sclerotia of a race of this fungus occurring even in a hot place like the Punjab State, can endure prolonged freezing without any impairment of their viability.

As will be communicated in a separate publication, the frozen sclerotia, though 100 per cent viable, were rendered sterile and incapable of producing apothecia.

(5) *Effect of wetting and drying:* Freshly harvested sclerotia from culture were divided into three lots. One lot was air-dried at room temperature; the second was wetted with excess of sterilized distilled water in a test-tube and was kept so for a fortnight continuously. The third lot was wetted and dried for a fortnight on alternate days, each alternate spell of wetting and drying being of 24 hours' duration. On testing, it was again observed that their viability was not at all impaired by such vicissitudes.

(6) *Effect of prolonged immersion of sclerotia in water at different temperatures:* Sclerotia were immersed in sterilized water in 150-ml. Erlenmeyer flasks and were placed at 5°, 10°, 15°, 20°, 25° and 30°C in constant-temperature cabinets. Their viability was tested at the end of two months and was found to be altogether unimpaired. Some sclerotia at 5° and 10°C, and all of them at 15° and 20°C, germinated giving out stipes, which, of course, could not produce apothecia under water.

(7) *Effect of flooding at different temperatures both under natural and sterilized conditions:* Sclerotia were placed on the surface of 50 gm. loam soil contained in 250-ml. Erlenmeyer flasks, which were flooded with water up to a depth of two inches. The experiment included two main series, one of which was arranged under aseptic conditions and was expected to contain the usual soil microflora. Each of these two main series was further subdivided into six lots of two each for distribution to temperatures of 5°, 10°, 15°, 20°, 25° and 30°C. The number of sclerotia used in each lot was 25.

The experiment was terminated at the end of five weeks and the results are given in Table IV.

The results show show that under sterile conditions, five weeks' flooding in loam soil does not at all damage the viability of sclerotia at any of the temperatures employed. At 15° and 20°C, all sclerotia even germinate and produce stipes. Under unsterilized conditions, it is only at 30°C that all the sclerotia lose their viability. At the temperature of 25°C and at the lower temperatures, their viability is the same as under sterile conditions.

TABLE IV. EFFECT OF FLOODING AT DIFFERENT TEMPERATURES IN LOAM SOIL ON THE VIABILITY OF SCLEROTIA UNDER STERILIZED AND UNSTERILIZED CONDITIONS, AS RECORDED AT THE END OF FIVE WEEKS

Temperature in °C	Under sterile conditions		Under unsterile conditions	
	Percentage viability of sclerotia	Remarks	Percentage viability of sclerotia	Remarks
5	100	No sclerotia germinated	100	No sclerotia germinated
10	100	65 per cent sclerotia germinated and gave out stipes	100	60 per cent sclerotia germinated and gave out stipes
15	100	All sclerotia germinated and gave out stipes	100	All sclerotia germinated and gave out stipes
20	100	All sclerotia germinated and gave out stipes	100	65 per cent sclerotia germinated and gave out stipes
25	100	No sclerotia germinated to produce stipes	100	No sclerotia germinated to produce stipes
30	100	No sclerotia germinated to produce stipes	0	80 per cent sclerotia rotted and disintegrated

The loss of their viability, rotting and disintegration at 30°C is attributed to the damaging effect of soil micro-organisms. A temperature of 30°C, though not actually lethal to this fungus, is decidedly not favourable to it either, and may be really accentuating the effect incidental to submergence of sclerotia under water, which, after all, is an unnatural environment. Sclerotia weakened by the combined effect of this high temperature and submergence under water may be more vulnerable to attack by soil organisms under unsterilized conditions.

Under sterile conditions, where all sclerotia remain viable even at 30°C when immersed, the combined effect of this high temperature and submergence under water is evidently not adverse enough to kill them in the absence of other organisms.

The lower temperatures, which may also be favourable to other soil organisms, are definitely favourable to this fungus and evidently help to maintain its sclerotia in a good condition, capable of resisting the inroads of other micro-organisms under unsterilized conditions. Their survival at lower temperatures under unsterilized conditions, therefore is not surprising.

(8) *Effect of different humidities:* Sclerotia put into cheese-cloth bags were suspended separately in one-quart Mason jars, in which were placed different proportions of sulphuric acid and distilled water to obtain humidities of 25, 50 and 75 per cent. Mason jars containing distilled water and concentrated sulphuric acid only furnished 100 per cent and zero per cent humidities, respectively. The jars were sealed securely and placed at room temperature (20°—25°C). The experiment was in triplicate and each replicate had 25 sclerotia. It was terminated at the end of one month. Tests showed 100 per cent viability of sclerotia at all the humidities tried.

The resistance of sclerotia to saturated atmosphere for a prolonged period is not in any way remarkable in view of their resistance to immersion under water for long periods. The other result of interest is the resistance of sclerotia to prolonged and severe desiccation as provided by concentrated sulphuric acid.

(9) *Effect of burial in manure at different temperatures both under sterile and unsterile conditions:* Sclerotia were put into 250-ml. Erelnmeyer flasks and were covered with moist, rotten barnyard manure. The experiment included two main series; in one of them sterilized and in the other unsterilized manure was used. Each series further consisted of six lots, which were distributed separately to temperatures of 5°, 10°, 15°, 20°, 25° and 30°C in constant-temperature cabinets. At the end of five weeks, the experiment was terminated and the viability of sclerotia was tested on agar. The results are presented in Table V.

TABLE V. EFFECT OF BURIAL OF SCLEROTIA IN MANURE, BOTH UNDER STERILE AND UNSTERILE CONDITIONS, AT DIFFERENT TEMPERATURES, AS RECORDED AT THE END OF FIVE WEEKS

Temperature in °C	Percentage viability of sclerotia	
	Under sterile conditions	Under unsterile conditions
5	100	100
10	100	100
15	100	100
20	100	84
25	100	36
30	0	0

All sclerotia buried in sterilized manure remained viable over a temperature range of 5° to 25°C, but the loss of their viability at 30°C is inexplicable. Under the supposedly sterile conditions, some micro-organisms, the presence of which, however, was not detected and suspected, may have grown imperceptibly in the manure on entering as contaminants from the air at the time of starting the experiment, and these may have invaded and killed the sclerotia at this high temperature, which is decidedly unfavourable to the latter. In the unsterilized series, the viability of sclerotia at the relatively low temperature range of 5° to 15°C remains unimpaired during the five-week period of the experiment, presumably due to the slow activity of the micro-organisms in the manure. Their damaging effect, however, becomes manifest at 20°C, and steadily mounts till at 30°C all the sclerotia are killed. All the sclerotia had rotted and disintegrated at this temperature.

(10) *Survival of sclerotia mixed with the seeds of gram* (Cicer arietinum *L.*)*:* Sclerotia produced in culture were mixed with seeds of gram in a cloth bag in September 1949, and were kept till March 1951 at room temperature ranging from 22° to 30°C, including summer months at St. Paul. Their viability was tested at about two-month intervals throughout this period. No deterioration in their viability was observed during the period of 18 months' storage. The transmission of the disease from year to year through sclerotia mixed with seed used for sowing is thus envisaged.

Biotic factors

Effect of other organisms: Sclerotia were mixed in moist sterilized and unsterilized soil contained in one-quart Mason jars and the cultures of *Fusarium* sp. (isolated from sclerotia obtained from the Punjab State), *Trichoderma lignorum*, *Aspergillus flavus* and *Bacillus subtilis* grown on potato broth were poured on the soil and incorporated into it. The experiment was run at the room temperature (22°—25°C) and was terminated at the end of three weeks. The results are given in Table VI.

Table VI. Effect of some other organisms on the viability of sclerotia mixed in sterilized and unsterilized moist soil at 22°-25°C, as recorded at the end of three weeks

Treatment	Percentage viability of sclerotia	
	Sterilized soil	Unsterilized soil
Sclerotia of the Punjab race plus *Fusarium* sp.	52	72
Sclerotia of the Punjab race plus *Trichoderma lignorum*	60	100
Sclerotia of the Punjab race plus *Aspergillus flavus*	0	0
Sclerotia of the Punjab race plus *Bacillus subtilis*	40	76
Sclerotia of the Punjab race alone	100	100

It is thus seen that *Aspergillus flavus* kills all the sclerotia, both in the sterilized and unsterilized soil. The effect of the remaining three organisms is more damaging in the sterilized than in the unsterilized soil, where, it seems, they have to compete with some other organisms, which may even be antagonistic to them. *Trichoderma lignorum* does not damage the sclerotia under unsterilized conditions, while it kills 40 per cent of them in sterilized soil where other organisms are absent and it has a free hand. Similarly, *Fusarium* kills 48 per cent of the sclerotia in sterilized and only 28 per cent in the unsterilized soil. Colonies of *Fusarium* sp. were obtained from dead sclerotia in most cases. It may be pointed out that sclerotia obtained from the Punjab had 94 per cent of their sclerotia invaded and killed by the *Fusarium* sp.

A similar behaviour is shown by *B. subtilis*, which also is more destructive under sterilized conditions.

Unfortunately, the experiment had to be terminated per force rather early, i.e., at the end of three weeks. Definite indications have been obtained that sclerotia of

this pathogen are vulnerable to attack by other organisms and they are in agreement with the views of other investigators. It may be possible to exploit the use of other organisms to destroy the sclerotia or at least decrease their number in the soil.

DISCUSSION

Sclerotia are extremely resistant to the action of chemicals, prolonged freezing under snow, freezing and thawing and wetting and drying, and these observations are in accord with those of other investigators. But their resistance to dry heat is amazing. In the writer's experiment, dry sclerotia endured six hours' continuous exposure to 60° and 70°C in the hot-air oven. Even at 80°C, 32 per cent of them survived a similar exposure. The survival of sclerotia in the Punjab during summer, where the temperature on the surface of the soil on bright sunny days in the afternoon at about 3 p.m. has been observed by the writer to touch 75°C mark, is explained by these tests.

Sclerotia, however, are extremely vulnerable to moist heat. A five minutes' immersion in hot water at 50°C is all that is needed to kill them. This easy vulnerability of sclerotia holds out much promise in the use of hot water as a means of killing them when mixed with the seeds of various crops. Pre-soaking of seed in cold water before hot-water treatment is not necessary and a five-minute exposure to hot water at 50°C will be harmless to most seeds.

The resistance of sclerotia to immersion in water is also very remarkable. In the present experiments they suffered no damage by two months' immersion in water at temperatures ranging from 5° to 30°C. This immersion, however, was in sterilized water. Decay of sclerotia within 23 to 45 days when flooded, as recorded by Moore (1949) in marl, muck or sandy soils, thus seems to be due to invasion by soil micro-organisms.

Sclerotia buried in compost heaps have no chance to survive, in the first place, due to the abundance of micro-organisms to the adverse effect of which they are extremely susceptible, and secondly, owing to the high temperatures that develop therein as a result of microbial activity. The writer has recorded temperatures as high as 50° to 55°C in the interior of compost heaps in the Punjab.

SUMMARY

The sclerotia of S. *sclerotiorum* are extremely resistant to the action of chemicals, and are not killed even by an exposure of one hour to one per cent solution of copper sulphate and sodium hypochlorite; can endure the effect of 0·1 per cent solution of mercuric chloride and one per cent solution of formalin for half an hour without any impairment of their viability; can even withstand absolute alcohol for a similar period; and their survival after a 10-minute soak in concentrated sulphuric acid is remarkable. This resistance is due to their hard, black protective rind.

They are also extremely resistant to dry heat; exposure at 60° and 70°C even for a period as long as six hours does not kill them; rather their exposure to 60°C for periods varying from half an hour to six hours, instead of damaging their viability, actually seems to stimulate them. In the present experiments, they produced vigorous

and fluffy cultures with large, and fully formed sclerotia much faster than the sclerotia not so treated.

The sclerotia are also very resistant to prolonged freezing, freezing and thawing, wetting and drying, and submergence under water. They are, however, extremely susceptible to the action of moist heat and are killed by an immersion of only five minutes in hot water at 50°C. They are also highly vulnerable to attack by other micro-organisms, particularly *Aspergillus flavus*, which kills its sclerotia both in the sterilized and unsterilized soil.

Sclerotia do not survive burial in a compost heap even for a few weeks at the relatively low temperature of 30°C, not to consider much higher temperatures of 50° to 55°C, which are readily obtained in the interior of compost heaps in the Punjab.

Sclerotia can live for long periods in a dry state, and the transmission of the disease through seed containing sclerotia used for sowing is envisaged.

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EFFECT OF CUTTING TREATMENTS ON THE YIELD AND CHEMICAL COMPOSITION OF GRASSLAND IN KUTCH

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Banni is a flat tract of grassland of 800 square miles lying along the north of Kutch. It is only two feet above sea-level with scanty rainfall. However, parts of the area are often flooded from the mainland of Kutch with monsoonal discharge, which is mixed with sea-water at the edges and the lower places. As a result, the state authorities have demarcated certain areas as saline land on coastal borders. The present investigation, which was suggested by Dr. R. O. Whyte, was undertaken with a view to compare the nutrient contents of various grasses in the tract and to ascertain the effect of harvesting at different stages of growth and frequencies of cutting on the composition of forage grasses.

EXPERIMENTAL

1. From the non-saline region of the tract, 58 samples of seven different grasses, viz., *dabhado* (*Desmostachya bipinnata* Stapff.), *dinai* (*Dichanthium annulatum*), *khivai* (*Sporobolus glaucifolius* Hochst.), *oin* (*Cressa cretica*), *samo* (*Echinochloa crus-galli*), *fuldi* (*Apluda aristata*), and *shialpunchh* (*Chloris montana*) were collected during the monsoon in 1954 and from the saline region seven samples of nine different grasses including seven varieties specified above and *khario* (*Aeluropus repens* (Desf.) Parl.) and *lamp* (*Aristida funiculata*) were collected and analyzed.

2. Thirty-two enclosures, each of 12 ft. $\times$ 8 ft. were laid at random around the eight representative villages—Bhojardo-V_1, Udai-Misariado-V_2, Hodka-V_3, Wadli-V_4, Wad-V_5, Adhiang-V_6, Gorewali-V_7, and Mithadi-V_8. Each enclosure was divided into three equal parts, A, B and C, and cuttings were taken as follows:

Method	Section			
I	A	August 24	September 24	October 24, 1956
II	B	—	September 24	October 24, 1956
III	C	—	—	October 24, 1956

In all, 192 samples of forage harvested from ground level were collected. The yields of foliage on fresh basis were recorded.

For the analysis of all the above samples, A.O.A.C. (1950) methods were followed. The pasture was harvested by the usual method and the grass was cut at a height of about 2-3 in. from the ground for chemical analysis and estimation of yield.

RESULTS AND DISCUSSION

All the results of chemical analysis are given on oven-dry basis.

TABLE I. COMPOSITION OF GRASSES FROM SALINE REGION OF BANNI AREA IN KUTCH

No. of samples analyzed	(11)		(12)		(8)		(10)		(7)		(4)		(8)		(11)		(5)	
	Dabhado		*Dinai*		*Khivai*		*Oin*		*Samo*		*Lamp*		*Shialpunchh*		*Khario*		*Fuldi*	
	Av.	S.E.	Av.	S.E.	Av.	S.E.	Av.	S.E.	Av.	S.E.	Av.	S.E.	Av.	S.E.	Av.	S.E.	Av.	S.E.
C. Protein	4·49	0·166	2·60	0·256	4·50	0·267	12·47	0·463	5·82	0·525	2·56	0·138	5·07	0·475	5·06	0·364	5·83	1·059
E. Extract	2·16	0·138	1·74	0·052	1·81	0·081	3·06	0·162	1·78	0·058	1·35	0·150	2·36	0·167	2·22	0·081	2·18	0·376
N.F.E.	49·86	0·716	47·76	0·499	49·16	0·923	41·42	1·254	45·28	0·705	52·10	5·240	48·61	0·815	47·21	0·858	48·38	0·997
C. Fibre	31·36	1·103	37·67	0·517	34·39	0·909	16·32	1·044	32·10	0·653	36·23	1·905	31·41	1·277	29·74	0·979	34·27	1·961
Ash	12·10	1·301	10·27	0·414	10·21	0·278	26·73	1·072	15·04	0·723	7·76	0·985	12·55	0·415	15·77	0·749	9·34	0·374
Silica	5·62	0·618	6·65	0·429	5·11	0·260	5·49	0·848	4·58	0·305	4·78	0·511	4·12	0·340	7·10	0·479	5·41	0·361
P	0·20	0·017	0·16	0·012	0·21	0·010	0·11	0·008	0·22	0·025	0·17	0·296	0·20	0·020	0·17	0·011	0·21	0·026
Ca	0·34	0·022	0·24	0·030	0·36	0·046	1·23	0·083	0·57	0·044	0·46	0·264	0·30	0·022	0·47	0·050	0·31	0·048

The composition of the grasses predominant in the saline region is given in Table I.

The results indicate that of all the forage plants, *oin* (*Cressa cretica* Linn.), which is a weed in the grassland, seems to be superior as it has 12·47 per cent crude protein and is least fibrous (16·32 per cent). All other grasses have only 2–6 per cent protein and crude fibre as high as 30–38 per cent. Moreover, *oin* is also quite rich in calcium, containing 1·23 per cent as against 0·24 to 0·57 in other grasses.

The composition of grasses from the non-saline region is almost similar to that of the same species of grasses from the saline region and therefore the results are not presented here.

These results clearly indicate that most of the grasses present in the grassland are poor in nutrients. The grassland has deteriorated considerably due to elimination of good varieties of grasses by over-grazing. It is possible that the potentiality of the grassland may be increased to an appreciable extent by rotational and controlled grazing.

Chemical composition of the forage harvested at different stages of growth:

The proportions of different varieties of grasses in all the enclosures are given in Table II and the nutrient contents of the forage when harvested at different stages of growth are given in Tables III—VI.

TABLE II. PROPORTION OF GRASSES IN ENCLOSURES (PER CENT)

Village		Enclosure			
		1	2	3	4
Bhojardo	V_1	D 70, Di 30	D 30, Di 70	Di 100	D 50, Di 50
Udai-Misariado	V_2	Di 100	D 25, Di 75	D 90, Di 10	D 80, Di 20
Hodka	V_3	L 100	L 100	L 100	S 100
Wadli	V_4	L 100	L 50, S 50	L 50, S 50	L 50, S 50
Wad	V_5	L 25, S 50, G 25	D 100	D 25, Di 50, S 25	D 25, L 75
Adhiang	V_6	L 25, S 75	L 25, S 75	L 100	G 100
Gorewali	V_7	D 50, Di 50	L 100	Di 70, S 15, Dr 15	L 75, S 25
Mithadi	V_8	D 40, Di 60	K 100	D 50, Di 50	Dr 20, Di 80

Name of the varieties of grasses. *Desmostachya bipinnata* Staff., *dabhado*—D; *Dichanthium annulatum* Staff., *dinai*—Di; *Aristida funiculata* Thin. and Rupr., *lamp*—L; *Alysicarpus rugosus* DC., *sonval*—S; *Eleusine flagellifera* Nees., *gandhiro*—G; *Sporobolus virginicus* Kunth., *drabh*—Dr; *Aeluropus repens* (Desf.) Parl., *khario*—K.

The composition of grasses harvested after varying period of growth differs significantly with respect to all the nutrients except crude protein (Table III). The ether extract and N.F.E. in the grasses at 11 weeks' growth are less than those in the seven-week-old grasses and they increase in the 15-week-old grasses where they attain their maximum

TABLE III. COMPOSITION OF FORAGE AT 7, 11 AND 15 WEEKS' GROWTH

(Average of four samples)

Age in weeks	C. Protein				E. Extract				N.F.E.			
	7	11	15	Average	7	11	15	Average	7	11	15	Average
V_1	7·44	6·60	5·97	6·67	2·00	2·04	2·29	2·11	45·88	46·12	51·18	47·73
V_2	6·20	5·01	4·76	5·32	2·05	1·90	2·35	2·10	48·86	47·79	51·22	49·29
V_3	7·82	6·77	9·08	7·89	2·55	1·81	2·40	2·25	44·43	47·90	49·83	47·39
V_4	6·05	7·13	9·19	7·46	2·16	1·46	1·99	1·87	49·22	47·58	48·22	48·34
V_5	6·49	7·19	6·01	6·56	1·89	1·94	2·24	2·02	47·88	45·71	51·31	48·30
V_6	5·90	6·74	9·42	7·35	1·88	1·61	2·23	1·91	50·93	46·79	50·34	49·35
V_7	5·21	5·84	5·24	5·43	1·98	1·69	2·09	1·92	49·95	46·69	51·44	49·23
V_8	4·64	4·80	5·02	4·82	2·01	1·78	2·41	2·07	51·50	45·56	49·64	48·90
Average	6·22	6·26	6·84	..	2·06	1·78	2·25	..	48·58	46·77	50·39	..

Age in weeks	C. Fibre				Phosphorus				Calcium			
	7	11	15	Average	7	11	15	Average	7	11	15	Average
V_1	34·28	36·03	30·90	33·47	0·17	0·15	0·12	0·15	0·31	0·69	0·63	0·54
V_2	33·02	35·37	30·79	33·06	0·14	0·12	0·10	0·12	0·27	0·56	0·45	0·43
V_3	32·41	34·55	28·58	31·88	0·15	0·13	0·13	0·14	0·54	0·76	1·05	0·78
V_4	33·91	36·27	28·72	32·97	0·11	0·10	0·12	0·11	0·33	0·77	0·94	0·68
V_5	32·55	34·47	29·42	32·15	0·15	0·15	0·12	0·14	0·42	0·79	0·69	0·63
V_6	32·08	34·14	27·64	31·29	0·13	0·12	0·13	0·13	0·37	0·55	0·86	0·59
V_7	32·34	36·73	31·76	33·61	0·16	0·14	0·11	0·14	0·39	0·59	0·43	0·47
V_8	31·18	35·42	31·36	32·65	0·15	0·14	0·13	0·14	0·33	0·62	0·40	0·45
Average	32·72	35·37	29·79	..	0·14	0·13	0·12	..	0·37	0·67	0·68	..

F. Value for	C. Protein	E. Extract	N.F.E.	C. Fibre	Phosphorus	Calcium
Villages	6·84*	3·49**	1·19	1·82	2·97**	7·92**
Cuttings	1·70	27·70**	18·21**	5·71**	6·88**	43·03**
C.D.						
Villages	1·00	0·18	..	..	0·017	0·101
Cuttings	..	0·11	1·00	0·86	0·003	0·062

*Significant at 5 per cent.
**Significant at 1 per cent.

values. The trend of variation in crude fibre is exactly opposite to that of ether extract and N.F.E. The phosphorus content decreases with maturity, while the calcium in the seven-week-old forage is only half of that in the older samples. The decrease in crude fibre at 15 weeks' growth may be attributed to the presence of certain species of grasses, which according to Phillips *et al.* (1954) show a drop in the content of this constituent at the most mature growth stages. The opposite trend of variation in phosphorus and calcium is in agreement with the observation reported by Ramiah (1933) and Krichgessner (1957).

The village-wise differences in the composition of forage are significant as far as protein, ether extract, phosphorus and calcium are concerned. Forage in village Mithadi (V_8) is the poorest in crude protein (4·82 per cent), while that from Hodka (V_3) is the richest in protein (7·89 per cent) and calcium (0·78 per cent).

TABLE IV. COMPOSITION OF CUTTING AFTER SEVEN WEEKS AND SUBSEQUENT MONTHLY CUTTINGS OF FORAGE

(Average of four samples)

Cutting	C. Protein				E. Extract				N.F.E.			
	7	11	15	Av.	7	11	15	Av.	7	11	15	Av.
V_1	7·44	7·67	7·85	7·65	2·00	1·76	1·92	1·89	45·88	47·41	46·97	46·75
V_2	6·20	6·88	5·86	6·31	2·05	1·67	1·82	1·85	48·86	47·80	47·57	48·08
V_3	6·50	9·12	8·89	8·17	2·39	1·97	2·38	2·25	47·88	45·14	45·06	46·03
V_4	5·90	11·14	10·43	9·16	1·88	2·14	2·38	2·13	50·93	44·88	47·78	47·83
Average	6·51	8·70	8·26	..	2·08	1·88	2·12	..	48·36	46·31	46·84	..

Cutting	C. Fibre				Phosphorus				Calcium			
	7	11	15	Av.	7	11	15	Av.	7	11	15	Av.
V_1	34·28	32·68	34·28	33·75	0·17	0·15	0·16	0·16	0·31	0·49	0·62	0·47
V_2	33·02	33·94	34·34	33·77	0·14	0·12	0·14	0·13	0·28	0·56	0·58	0·47
V_3	32·55	34·52	33·27	33·45	0·16	0·19	0·16	0·17	0·42	0·57	0·49	0·49
V_4	32·08	31·34	29·80	31·07	0·13	0·18	0·13	0·15	0·37	0·71	0·51	0·53
Average	32·98	33·12	32·92	..	0·15	0·16	0·15	..	0·34	0·58	0·55	..

	C. Protein	E. Extract	N.F.E.	C. Fibre	Phosphorus	Calcium
F Value for						
Villages	6·05	8·99**	1·75	2·73	5·33**	0·08
Cuttings	7·62	10·60**	2·91	0·09	1·41	2·39
C.D.						
Villages	..	0·18	..	..	0·02	..
Cuttings	..	0·16	..	..	..	..

**Significant at 1 per cent.

The composition of the first cut of grasses at seven weeks and of subsequent monthly aftermath cuts was studied.

The composition of the different cuttings of forage does not vary significantly except in ether extract (Table IV). It is only 1·88 per cent in the first aftermath growth as compared to 2·10 per cent in the other cuts. It is interesting to note that the second and the third cuttings in almost all the villages are richer in crude protein than the first, although the difference is not statistically significant. It is due to the fact that the first cutting is older than the later two cuttings. Similar effect of maturity on protein content was observed by Staples *et al.* (1951) in South Dakota prairie hays. The village-wise variation in the composition of grasses is not very significant. Another type was to harvest the plot first after 11 weeks and then one more aftermath cut at four weeks' interval. The results are given in Table V.

TABLE V. COMPOSITION OF FORAGE AT 11 WEEKS' GROWTH AND FOUR WEEKS' AFTERMATH GROWTH

(Average of four samples)

	C. Protein			E. Extract			N.F.E.		
Cutting	11	15	Average	11	15	Average	11	15	Average
V_1	6·60	6·82	6·71	2·04	2·60	2·32	46·12	46·98	46·55
V_2	5·01	4·71	4·86	1·90	2·55	2·22	47·80	48·72	48·28
V_3	6·78	8·42	7·60	1·82	2·17	2·00	47·91	46·78	47·34
V_4	7·13	8·71	7·92	1·46	2·48	1·97	47·58	47·13	47·36
V_5	7·19	8·71	7·95	1·94	2·62	2·28	45·71	48·80	47·26
V_6	6·76	10·18	8·47	1·61	2·47	2·04	46·79	48·92	47·86
V_7	5·84	6·56	6·20	1·70	2·54	2·12	46·69	50·80	48·74
V_8	4·80	8·22	6·51	1·78	2·78	2·28	45·56	47·22	46·39
Average	6·26	7·78	..	1·78	2·53	..	46·77	48·17	..

	C. Fibre			Phosphorus			Calcium		
Cutting	11	15	Average	11	15	Average	11	15	Average
V_1	30·03	34·27	35·15	0·16	0·12	0·14	0·69	0·60	0·64
V_2	35·37	34·35	34·86	0·12	0·11	0·12	0·56	0·46	0·51
V_3	34·56	30·33	32·44	0·13	0·14	0·13	0·76	0·78	0·77
V_4	36·27	32·92	34·60	0·10	0·12	0·11	0·78	0·84	0·81
V_5	34·46	30·45	32·46	0·15	0·16	0·15	0·80	0·59	0·70
V_6	34·14	29·88	32·01	0·12	0·14	0·13	0·55	0·76	0·66
V_7	36·73	30·95	33·84	0·14	0·16	0·15	0·60	0·40	0·50
V_8	35·41	29·01	32·21	0·14	0·21	0·18	0·62	0·49	0·56
Average	35·37	31·52	..	0·13	0·14	..	0:67	0·62	..

	C. Protein	E. Extract	N.F.E.	C. Fibre	Phosphorus	Calcium
F Value for						
Villages	5·90**	1·77	0·74	2·47*	4·23**	5·12*
Cuttings	15·20**	100·06**	3·74	42·84**	2·68	2·20
C.D.						
Villages	1·42	..	..	2·43	0·03	0·15
Cuttings	0·71	0·15	..	1·21	..	..

*Significant at 5 per cent. **Significant at 1 per cent.

Aftermath growth is richer in all the nutrients than the forage harvested at 11 weeks' age. The former, being tender, has 7·78 per cent crude protein and 31·52 per cent crude fibre as against 6·26 and 35·37 per cent respectively in the latter. The high protein content in the aftermath growth is in agreement with the observation reported by Agerberg (1956).

The composition of grasses varies significantly from village to village with respect to crude protein, crude fibre and minerals. Crude protein in the grasses from Udai-Misariado (V_2) is minimum (4·86 per cent), while the crude fibre content of grasses from Bhojardo (V_1), Udai-Misariado (V_2) and Wadli (V_4) (about 35 per cent) is higher than that of grasses from the remaining villages (about 32 per cent). It is remarkable that the grass from Wadli (V_4) contains only 0·11 per cent phosphorus as against 0·18 per cent in the grass from Mithadi (V_8), while the calcium content in the former is high (0·81 per cent) and in the latter it is low (0·56 per cent). These results bring out very well the antagonism between phosphorus and calcium contents.

Comparison of the composition of the four weeks' aftermath cuts after initial cut at 7 and 11 weeks in Table IV and V indicates not much variation except in the case of ether extract which was higher (2·33 per cent) in the latter as compared to only 1·88 per cent in the former.

From the above observations with regard to the variation in the composition of the grass harvested at different stages of growth, it is clear that the yield data should be simultaneously considered while deciding the best method of harvest. The yields of forage at three different intervals of harvest are given in Table VI.

TABLE VI. FORAGE YIELDS AT THREE DIFFERENT INTERVALS OF HARVEST

(gm. on fresh basis)

Interval of cutting		I	II	III	Total	Average
Village	Enclosure No.					
Bhojardo	1	1,476	795	1,362	3,633	1,211
	2	3,064	2,497	1,362	6,923	2,308
	3	3,745	2,497	2,270	8,512	2,837
	4	1,362	1,249	1,022	3,633	1,211
		9,647	7,038	6,016	22,701	1,892
Udai-Misariado	5	1,022	795	908	2,725	908
	6	2,043	908	1,362	4,313	1,438
	7	3,178	2,043	1,362	6,583	2,194
	8	2,383	1,135	1,362	4,880	1,627
		8,626	4,881	4,994	18,501	1,542
Hodka	9	811	1,271	1,362	3,444	1,148
	10	1,149	922	908	2,979	993
	11	724	960	681	2,365	788
	12	2,514	934	908	4,356	1,452
		5,198	4,087	3,859	13,144	1,095

TABLE VI—*Contd.*

Village (Interval of cutting)	Enclosure No.	I	II	III	Total	Average
Wadli	13	1,148	698	908	2,754	918
	14	1,249	908	681	2,838	946
	15	1,862	925	681	3,468	1,156
	16	1,566	965	681	3,212	1,071
		5,825	3,496	2,951	12,272	1,023
Wad	17	1,612	1,391	908	3,911	1,304
	18	4,880	3,405	2,951	11,236	3,745
	19	2,611	1,930	2,724	7,265	2,422
	20	2,724	2,497	1,816	7,037	2,346
		11,827	9,223	8,399	29,449	2,454
Adhiang	21	2,060	1,839	1,362	5,261	1,754
	22	1,930	1,249	1,362	4,541	1,514
	23	1,158	473	1,362	2,993	998
	24	1,105	1,391	908	3,404	1,135
		6,253	4,952	4,994	16,199	1,350
Gorewali	25	1,476	1,136	1,362	3,974	1,325
	26	1,259	715	908	2,882	961
	27	1,476	908	1,362	3,746	1,249
	28	1,033	698	908	2,639	880
		5,244	3,457	4,540	13,241	1,103
Mithadi	29	2,384	1,362	1,135	4,881	1,627
	30	818	579	454	1,851	617
	31	2,270	1,362	1,816	5,448	1,816
	32	1,589	1,249	1,135	3,973	1,324
		7,061	4,552	4,540	16,153	1,346
Total		59,681	41,686	40,293	1,41,660	..
Average		1,865	1,303	1,259	1,476	..

	F. Value	Critical difference at 5 per cent
Interval of cutting	26·548**	182
Between villages	20·621**	297
Within villages	6·762**	583

Statistical analysis of the data reveals that the interval of cutting has a significant effect on the yield of forage. The maximum yield of 1,865 gm. per plot (5,595 lb. per acre) is obtained when the forage is first harvested after about two months and then at monthly intervals. This yield is 45 per cent higher than that obtained by the other two intervals of harvest. There is no significant difference between the yield obtained by the latter two intervals. However, when the forage is harvested after about three months' growth and again after a month, the yield tends to be higher than when it is harvested only once after about four months. These results are similar

to the findings of Patel *et al.* (1950), who have reported that the yield of guinea-grass, napier-grass and elephant-grass cut at intervals of 30 days is higher than when cut at intervals of 50 or 70 days.

The yield of grass per acre, calculated on the basis of the total average yield from an enclosure, varies from village to village to a considerable extent. The maximum yield of 2,454 gm. per plot (7,362 lb. per acre) is obtained in the village Wad, while the minimum of 1,023 gm. per plot (3,068 lb. per acre) in Wadli, the average being 1,476 gm. per plot (4,427 lb. per acre) for the entire area. If, however, the best period of harvest is adopted all over the area, the average yield would be increased by about 40 per cent.

Within enclosures of the same village there are highly significant differences in yield. This may be attributed to the prevalence of different species of grasses and their uneven distribution over the area. The differences in the yield of forage in various villages may also be due to the same factor.

SUMMARY

Studies on the forage plants of Banni area in Kutch has revealed that *dabhado* (*Desmostachya bipinnata* Stapf.) and *dinai* (*Dichanthium annulatum* Stapf.) are the grasses occurring to the extent of about 50 per cent each in the villages Bhojardo, Udai-Misariado, Gorewali and Mithadi. In other four villages, Adhianag, Wad, Hodka and Wadli, *lamp* (*Aristida funiculata*, Trin. & Rupra.) mixed with other grasses is predominant. From the chemical studies it is clear that protein content of these grasses varies from 2—6 per cent while crude fibre is as high as 30—38 per cent. *Oin* (*Cressa cretica* Linn.), having 12·47 per cent protein and 16·32 per cent crude fibre, is the best forage species in both the saline and non-saline regions, though its occurrence is scanty.

With increase in growth period from 7 to 15 weeks, there is a significant rise in calcium from 0·37 to 0·68 per cent. Forage harvested at 15 weeks' growth is the richest in nutrients, having 2·25 per cent ether extract 50·39 per cent N.F.E. and only 29·79 per cent crude fibre.

When the forage is first cut at seven weeks' age and then at monthly intervals, it is observed that except in case of ether extract, there is no significant difference in the composition of different cuts; however, when the forage is first cut at 11 weeks' age and then at monthly aftermath growth, the latter is the richest in all the nutrients.

Taking into consideration the chemical composition and the forage yields, it appears that the best stage of harvest would be to take the first cutting after about two months' growth followed by subsequent monthly cuttings which has yielded 5,595 lb. of forage per acre, i.e., about 45 per cent higher yield than that obtained by harvesting at other intervals.

Yield and composition of forage in various villages, varied significantly probably because of prevalence of different species of grasses and their uneven distribution.

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NITROGEN FIXATION BY *AZOTOBACTER* SPECIES AT HIGH ALTITUDES

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Soils at high altitudes are generally characterized by high contents of nitrogen. In spite of the proverbially low content of nitrogen of Indian soils, soils from hilly areas have been observed to contain unusually large quantities of nitrogen (Taylor *et al.*, 1935; Hoon, 1939; Mukherjee and Das, 1942). Increase in the nitrogen content of soils at altitude has also been observed elsewhere (Sievers and Holtz, 1923; Dean, 1930; Hockensmith and Tucker, 1933; Hordon, 1936). While climatic factors like low temperature and high rainfall may account for accumulation of nitrogen in soils at higher altitudes (Jenny, 1941), it would be interesting to know the part played by the nitrogen-fixing microbial population in the nitrogen economy of soils at high altitudes.

During the search for some efficient strains of *Azotobacter* spp. for inoculation purposes, soils at high altitudes were observed to be the habitats of a large number of the strains having proportionately high nitrogen-fixing capacity. The results of analysis of the data pertaining to the composition of these soils have been reported elsewhere (Raychaudhuri and Sen, 1957). The present report deals with the study of the nitrogen-fixing capacity of *Azotobacter* spp. in relation to the elevation of the habitat. The soils were collected from Mussoorie hills in Uttar Pradesh.

MATERIALS AND METHODS

Soils: A description of the soils, used in the present study is given below.

Sample Number	*Description*
6	At base of ridge, wheat plots, levelled in paddy field fashion, plants 6 in. high, showing signs of wilting, elevation 1,890 ft., organic nitrogen 0·074 per cent, pH 6·5
7	Kaulagarh Tea Garden, signs of manuring, elevation 2,210 lb., organic nitrogen 0·131 per cent, pH 6·2
29	5·7 miles from Dehra Dun, south on the left side of the road, out hills nearby, slope 6, forest floor 1—2 in., yellow rocky soil, elevation 3,300 ft., organic nitrogen 0·115 per cent, pH 5·6
28	5·7 miles from Dehra Dun, on top of little knoll, Sal (*Shorea robusta*) trees very uniform in size, 28 ft. high, 5 ft. in diameter, forest floor ½—1 in., south-west slope 12—15, elevation 3,400 ft., organic nitrogen 0·135 per cent, pH 5·9
42	Lowest cultivated field, slope 15, elevation 4,050 ft., organic nitrogen 0·226 per cent, pH 6·3

Sample Number	*Description*
41	Highest rice patch with cattle grazing on it, level, elevation 4,070 ft., organic nitrogen 0·191 per cent, pH 7·7
39	13 miles to Mussoorie, about 20 ft. below road, grass and bush, south-east exposure, slope 31, elevation 4,430 ft., organic nitrogen 0·331 per cent, pH 6·8
27	Pasture, slope 10, elevation 4,750 ft., organic nitrogen 0·134 per cent, pH 8·0
25	Below village Rtarkuli, near isolated huts, slope 45, undisturbed spot near agave, elevation 5,000 ft., organic nitrogen 0·351 per cent, pH 7·6
23	Below Krishan temple, gravelly, wheat spot, wheat following rice, elevation 5,400 ft., organic nitrogen 0·236 per cent, pH 7·4
22	Below Krishan temple, level, terraced, gravelly, no manure, elevation 5,470 ft., organic nitrogen 0·247 per cent, pH 7·0
15	Kapliarni Khala creek, old patch of wheat, containing a little manure, elevation 5,730 ft., organic nitrogen 0·131 per cent, pH 7·8
14	Above the road near Mussoorie, pasture, dark grey-brown soil, elevation 6,280 ft., organic nitrogen 0·271 per cent, pH 7·2

Isolation of Azotobacter: Every hundred ml. of sterile Ashby's mannite solution (Ashby, 1907) was inoculated with one gm. of each soil. A loopful of the culture liquid after incubation for a week at 32·5°C was plated in several dilutions in mannite agar. The colonies developing after five days were examined and the material transferred to mannite agar slants for study of morphological, cultural and biochemical characteristics and for determination of the nitrogen-fixing capacities. The number of strains isolated from the soils on the basis of colony characteristics (size, appearance and pigmentation) were two from soil No. 6, one each from 7, 29 and 28, three each from 42 and 15, four each from 41, 39, 25, 23 and 14, and five each from 27 and 22.

Nitrogen-fixing capacity: Nitrogen-fixing capacities of the pure strains were determined in Jensen's sucrose-agar medium (Jensen, 1951) after incubation for two weeks.

OBSERVATIONS

Nitrogen-fixing capacities of the different strains of *Azotobacter* spp. from the soils at different altitudes are given in Table I.

DISCUSSION

There were differences in the nitrogen-fixing capacities of different strains of *Azotobacter* spp. occurring in the same altitude. Maximum nitrogen-fixing capacity was observed in the case of some strains isolated from an altitude of 4,750 ft. The nitrogen-fixing capacities of the strains, however, decreased thereafter. The nitrogen-fixing capacity of the organism increased as the logarithm of the elevation [r(N.F.C.) $(\log_{10}H) = +0{\cdot}36$]. The relationship between the N.F.C. and altitude could be noted in the expression $N.F.C. = 8{\cdot}49 \log_{10}H$, where N.F.C. is the nitrogen-fixing capacity in mg. per gm. of sucrose during 14 days and H is the altitude in 100 ft. (Fig. 1).

TABLE I. NITROGEN-FIXING CAPACITIES OF *AZOTOBACTER* SPP. IN SOILS OCCURRING AT DIFFERENT ELEVATIONS IN THE MUSSOORIE HILLS

(Figures of nitrogen are quoted after deduction of blanks)

Soil sample	Height (ft.)	Strain	Mg. of nitrogen fixed per gm. of sucrose — Average of three replicate determinations
6	1,890	(i)	10·9 ± 0·07
		(ii)	12·9 ± 0·35
7	2,210	(i)	8·7 ± 0·18
29	3,300	(i)	7·3 ± 0·69
28	3,400	(i)	12·5 ± 0·27
42	3,870	(i)	16·0 ± 0·15
		(ii)	15·9 ± 0·17
		(iii)	9·7 ± 0·48
41	4,070	(i)	10·5 ± 0·78
		(ii)	14·6 ± 0·53
		(iii)	14·6 ± 0·23
		(iv)	19·3 ± 0·66
39	4,430	(i)	14·9 ± 0·97
		(ii)	15·7 ± 0·69
		(iii)	15·8 ± 0·95
		(iv)	13·7 ± 0·98
27	4,750	(i)	16·7 ± 0·13
		(ii)	16·5 ± 0·87
		(iii)	13·6 ± 1·42
		(iv)	18·2 ± 0·27
		(v)	18·0 ± 0·31
25	5,000	(i)	18·3 ± 0·68
		(ii)	10·9 ± 0·39
		(iii)	16·5 ± 0·37
		(iv)	13·8 ± 1·48
23	5,400	(i)	12·9 ± 1·04
		(ii)	16·7 ± 0·69

TABLE I—*Concld.*

Soil sample	Height (ft.)	Strain	Mg. of nitrogen fixed per gm. of sucrose — Average of three replicate determinations
		(iii)	10·7 ± 0·66
		(iv)	15·9 ± 0·18
22	5,550	(i)	12·9 ± 0·63
		(ii)	13·3 ± 0·29
		(iii)	14·0 ± 0·31
		(iv)	13·7 ± 0·37
		(v)	15·8 ± 1·31
15	5,730	(i)	15·4 ± 1·03
		(ii)	14·4 ± 0·40
		(iii)	12·8 ± 0·23
14	6,280	(i)	12·4 ± 0·61
		(ii)	11·5 ± 0·35
		(iii)	11·8 ± 0·11
		(iv)	15·5 ± 0·49

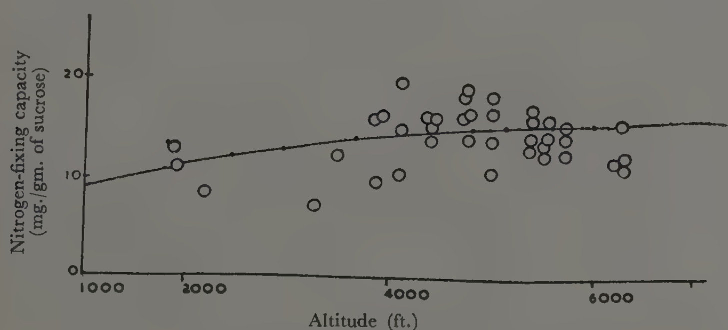

FIG. 1. NITROGEN-FIXING CAPACITY OF *Azotobacter* SPP. IN RELATION TO THE ALTITUDE OF THE HABITAT

Burk (1930) observed that the efficiency of nitrogen fixation by *Azotobacter* increased with decreased pO_2 which could be observed with increasing altitude. Similar observations were made by Garbosky (1957). It was not possible to know if the organism, subjected to long continued low pO_2 at higher altitudes, had developed

greater efficiency of nitrogen fixation. It was observed, however, that apart from low decomposition of organic matter at higher altitudes, the greater efficiencies of the nitrogen-fixing organisms like *Azotobacter* might, at least partly, be responsible for the higher nitrogen content at higher altitudes.

There was no deterioration of the efficiencies of the strains when they were brought under normal atmospheric pressure. Some of these strains like A. 23(II), A. 25(I), A. 41(IV), have been known to retain their nitrogen-fixing capacity even after continuous cultivation for five years in artificial media in the laboratory.

SUMMARY

During the study of the nitrogen-fixing capacities of *Azotobacter* spp. occurring at different altitudes under the same climatic conditions, it was observed that the nitrogen-fixing capacity of the organism varied as the logarithm of the altitude.

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INVESTIGATION ON THE DETERMINATION OF AVAILABLE COBALT IN SOILS OF KAIRA DISTRICT IN GUJARAT*

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Of the four forms of soil cobalt, viz., (i) water-soluble, (ii) easily exchangeable or replaceable, (iii) dilute acid-soluble and (iv) non-replaceable, the water-soluble cobalt is the most readily available form. But this form is present in soils in extremely minute quantities (Riche, 1959). The bulk of soil cobalt is in non-replaceable form. Mitchell (1955) is of the opinion that the total content can be a reasonable indication of trace-element status of a soil. But, Lyon *et al.* (1952) have pointed out that the total contents of trace elments throw no light on availability to plant, though they may be of interest from the geo-chemical aspect. The available nutrient status of a soil is generally judged by its water-soluble and exchangeable contents as determined by leaching with solutions of reagents. Among the various extractants employed for available cobalt are: (i) decinormal hydrochloric acid, (ii) 2·5 per cent acetic acid adjusted to pH 2·5, (iii) 2·5 per cent acetic acid adjusted to pH 4·5, and (iv) neutral normal ammonium acetate. As no satisfactory laboratory method applicable to all soils is available to estimate the amounts of soil cobalt available to plants, it was thought necessary to carry out a greenhouse experiment by growing plants in representative soils and to correlate the total plant uptake with that extracted by different solutions and to follow that particular extraction method which gave the highest correlation with that of cobalt taken up by plants.

MATERIAL AND METHODS

To find out an extractant suitable for the estimation of available cobalt in the soils, a greenhouse experiment was conducted using *jowar* (*Sorghum vulgare*) as the indicator plant. Twenty-one samples of typical soils of Kaira district were collected and filled in china-clay pots, seven inches in height and five inches in diameter with a half-inch drainage hole at the bottom. All the pots were filled at the bottom with a two-inch layer of acid-washed gravel. The experiment was carried out in four replications. Eight seeds of *jowar* were sown in each pot and when the plants were nine days old, they were thinned to four in each pot. The plants were allowed to grow for eight weeks and the portions of the plants above the ground were harvested. After drying at 70° C for 48 hours in an electric oven, the samples were cut into small pieces and powdered. Cobalt was estimated from the samples colorimetrically by 2-nitroso-1-naphthol method (Clark, 1958) after digesting the samples by Sandell's (1950) method.

*Partly based on the thesis submitted by K. G. Reddy for M.Sc. degree of Sardar Vallabhbhai University, Anand.

Soils studied

The soils studied represented the important types of the tract, viz., (i) *goradu*, (ii) *besar*, (iii) *kyari*, and (iv) *bhatha*. Texturally they are all loams containing large proportions of fine sand. The first two are coarser and contain clay from six to ten per cent and sand from 70 to 80 per cent. The other two are finer and contain 10 to 13·5 per cent of clay and 50 to 70 per cent of sand. Their pH varies from 7·2 to 8·6, and $CaCO_3$ content from nil to 6·69 per cent (Table I).

TABLE I. COMPOSITION OF SOILS OF KAIRA DISTRICT

Village	Total soluble salts per cent	pH	Coarse and fine sand per cent	Silt and clay per cent	$CaCO_3$ per cent	Organic matter per cent	Total nitrogen per cent	Total P_2O_5 mg./100 gm. soil
Balasinor	0·05	8·2	84·5	13·9	0·4	1·1	0·067	252·6
Bamanva	0·02	7·2	87·9	11·4	0·1	0·5	0·046	66·6
Bandhani	0·03	8·6	85·5	13·4	0·2	0·7	0·052	172·0
Bhadran	0·05	7·7	74·1	23·4	0·7	1·7	0·120	333·2
Borsad	0·03	7·8	75·5	23·3	0·1	0·9	0·056	193·7
Chakalasi	0·03	8·4	84·3	14·8	0·0	0·8	0·050	83·7
Dharmaj	0·06	7·8	74·3	24·7	0·1	0·8	0·049	96·1
Kapadvanaj	0·02	8·5	89·6	8·4	1·3	0·6	0·042	117·8
Kaira	0·01	8·4	47·7	44·9	6·0	1·5	0·091	122·4
Kherda	0·04	8·3	48·1	38·8	2·2	1·8	0·092	134·8
Anand Institute (*Goradu*)	0·02	7·5	82·6	16·7	0·0	0·6	0·042	86·8
Anand Institute (*Kyari*)	0·04	8·2	63·1	35·6	0·0	1·2	0·073	187·5
Limbasi	0·05	7·9	51·4	40·9	6·6	0·9	0·049	156·5
Mahudha	0·01	7·9	66·8	31·3	0·5	1·4	0·092	198·4
Nayaka	0·04	8·5	66·6	31·8	0·5	0·9	0·062	89·9
Radhavanaj	0·03	7·4	73·5	35·3	0·2	0·9	0·062	122·4
Sansoli	0·03	8·6	63·2	30·3	5·4	0·9	0·070	113·1
Thasara	0·07	8·3	80·8	18·2	0·2	0·6	0·050	241·8
Vanthavali (*Goradu*)	0·02	7·2	84·2	14·9	0·2	0·6	0·041	43·4
Vanthavali (*Kyari*)	0·01	7·7	70·6	27·9	0·3	1·2	0·059	137·9
Vasad	0·05	8·3	85·0	13·8	1·1	0·9	0·043	83·8

Extraction of cobalt in soils by different reagents

The soil samples were analyzed in the laboratory for their available cobalt by extracting with the following reagents:

(a) decinormal hydrochloric acid (Pickett and Dinius, 1954).

(b) 2·5 per cent acetic acid (pH 2·5) (Pickett and Dinius, *loc. cit.*).

(c) 2·5 per cent acetic acid (pH 4·5). Extraction was done as in method (b).

(d) normal ammonium acetate of pH 7·0 (Carringham and Erwin, 1950).

RESULTS AND DISCUSSION

Table II shows the uptake of cobalt by *jowar* plants and available cobalt extracted by the above four different extractants. Out of the four extractants, normal neutral ammonium acetate was found to extract the minimum amount of cobalt. The amounts extracted are definitely more in acid extractants, and as the acidity increases, the amounts extracted also increase. The amounts extracted by 0·1N HCl are the

TABLE II. CORRELATION BETWEEN THE UPTAKE OF COBALT BY *JOWAR* AND EXTRACTABLE COBALT FROM SOILS

Place	Texture	Uptake by *jowar* plants per pot (mmg.)	Extractable cobalt in ppm. 0·1N HCl	2·5% acetic acid pH 2·5	2·5% acetic acid pH 4·5	Normal ammonium acetate pH 7·0
Balasinor	Sandy loam	1·692	2·88	0·390	0·187	0·069
Bamanva	Fine sandy loam	1·862	3·47	0·388	0·200	0·040
Bandhani	Fine sandy loam	3·006	2·45	0·548	0·150	0·050
Bhadran	Silty clay loam	1·686	0·55	0·100	0·039	0·009
Borsad	Fine sandy loam	1·728	2·00	0·332	0·070	0·027
Chacklasi	Sandy loam	1·224	1·36	0·195	0·087	0·029
Dharmaj	Sandy loam	2·500	2·52	0·752	0·270	0·047
Kapadavanj	Coarse sandy	2·888	2·26	0·652	0·336	0·065
Kaira	Clayey	1·116	2·69	0·208	0·062	0·016
Kherada	Clayey	3·924	6·94	0·920	0·110	0·043
Anand Institute (*Goradu*)	Fine sandy loam	2·754	2·90	0·668	0·296	0·067
Anand Institute (*Kyari*)	Clay loam	1·548	4·60	0·344	0·067	0·017
Limbasi	Silty clay loam	2·448	2·81	0·444	0·233	0·048
Mahudha	Clay loam	1·448	2·81	0·180	0·060	0·022
Nayaka	Silty clay loam	0·212	3·01	0·192	0·040	0·020
Radhavanaj	Clayey	2·322	3·18	0·720	0·317	0·085
Sansoli	Sandy loam	3·240	3·92	0·320	0·110	0·020
Thasra	Sandy loam	2·106	1·96	0·456	0·300	0·053
Vanthavali (*Goradu*)	Sandy loam	1·686	3·01	0·360	0·070	0·016
Vanthavali (*Kyari*)	Fine sandy loam	2·862	1·84	0·564	0·347	0·089
Vasad	Fine sandy	2·109	5·12	0·720	0.093	0·026
Correlation coefficient "r"			+0·3635 N.S.	+0·7347**	+0·5058*	+0·4901*

**Significant at 1% level. *Significant at 5% level.

maximum and are, in all cases, more than 30 times the amounts obtained with neutral ammonium acetate. In the decreasing order of the amounts extracted, the reagents can be arranged as:

decinormal hydrochloric acid > 2·5 per cent acetic acid at pH 2·5 > 2·5 per cent acetic acid at pH 4·5 > normal ammonium acetate at pH 7.

These results are in conformity with those obtained by Bannerjee *et al.* (1953), who stated that decinormal hydrochloric acid extracted much more cobalt than did 2·5 per cent acetic acid.

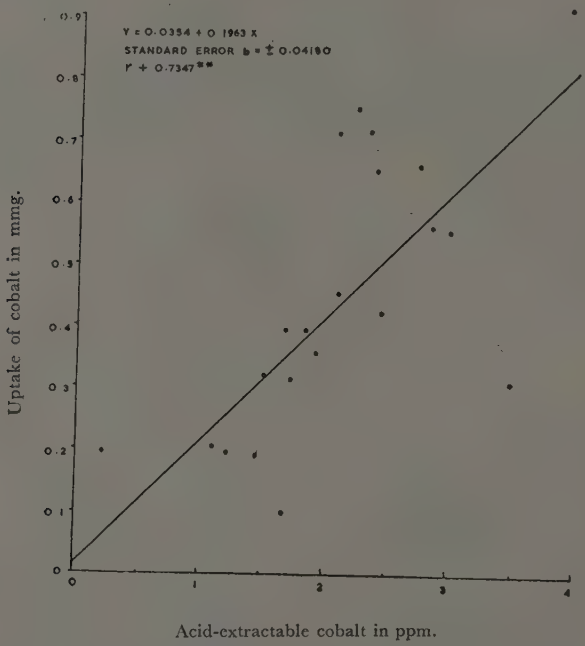

FIG. 1. RELATION BETWEEN THE UPTAKE OF COBALT BY *jowar* PLANT AND AMOUNT OF COBALT EXTRACTED BY 2·5 PER CENT ACETIC ACID (pH 2·5)

Statistical analysis of the data showed that there is a positive correlation of the amounts extracted with any of the four reagents with the uptake of cobalt by *jowar* plants; but in the case of decinormal hydrochloric acid, there is no significant relationship—the correlation coefficient being +0·3635. The remaining three reagents show a significant relationship—the correlation coefficients being + 0·4901, + 0·5058 and + 0·7347 for neutral normal ammonium acetate, 2·5 per cent acetic acid at pH 4·5, and 2·5 per cent acetic acid at pH 2·5 respectively. Thus, the highest correlation (significant at one per cent level) is given by the amount of cobalt extracted by 2·5 per

cent acetic acid at pH 2·5 with the uptake of cobalt by plants. The equation of the regression line minimizing the Y components of derivation is: $Y=0{\cdot}0354+0{\cdot}1963X$ shown in Fig. 1, and its significance is tested by calculating the F ratio. The observed value (22·07) is seen to be much larger than F at 1 per cent (8·18) for 1 and 19 degrees of freedom. This showed the suitability of 2·5 per cent acetic acid of pH 2·5 for extracting the available cobalt from the soil samples. Mitchell *et al.* (1941) also found this reagent suitable for determining available cobalt in soils, while Bannerjee *et al.* (*loc. cit.*) have shown it to be useful for diagnostic purposes in cobalt deficiency in pastures.

SUMMARY

To find a suitable method for determining the availability of cobalt in soils of Kaira district in Gujarat, the amounts extracted from 21 samples of representative soils by neutral normal ammonium acetate, 2·5 per cent acetic acid adjusted to pH 4·5, 2·5 per cent acetic acid adjusted to pH 2·5 and decinormal hydrochloric acid, were correlated with the uptake of cobalt by *jowar* plants grown in the same soils in pots. Extractable cobalt by 2·5 per cent acetic acid adjusted to pH 2·5 gave the highest correlation and hence this method appears to be the most suitable for determining the amount of available cobalt in these soils.

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EFFECT OF SOAKING SEEDS IN NUTRIENT SALTS

II. ABSORPTION OF ZINC BY WHEAT SEEDS FROM ZINC SULPHATE SOLUTION AND ITS INFLUENCE ON GROWTH AND YIELD

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The results on chemical estimations of zinc ions absorbed by cotton seeds from zinc sulphate solution, their performance under water culture deficient in zinc and also under field conditions, were reported earlier (Singh, 1961). It was concluded that the extra amount of zinc absorbed by the cotton seeds during soaking helped in maintaining the growth for a longer period under zinc deficiency, as compared to unsoaked controls, but was not sufficient for the entire life-cycle of the plant. Under field conditions, soaking the seeds in zinc solution had no influence on the yield of seed cotton.

In the present paper, the results on the amount of zinc absorbed by wheat seeds from zinc sulphate solution, their performance under water culture deficient in zinc and also under field conditions are presented.

MATERIAL AND METHODS

Wheat grains of variety N.P. 718 were soaked for 24 hours at room temperature in various concentrations of zinc sulphate solution (M/100 to M/1000) in a ratio of 1:10 between the weight of the seed and the volume of the solution in June, 1958. The seeds, before soaking, were sterilized with one per cent mercuric chloride solution; after soaking they were rinsed with distilled water, and later germinated on moist filter paper in glass petri dishes. The seedlings were separated from the endosperm, when three to four days old. The zinc content of the seedlings and endosperm was estimated separately by the polarographic method, adopting the method of Reed and Cummings (1944). The amount of zinc left in the soaking solution after removing and washing the seeds was also estimated just to determine the total quantity of zinc absorbed by the seeds from the solution. The effect of zinc concentration on the germinating capacity of the seeds was also observed. The seeds under control were soaked in distilled water only.

Water-culture experiment: In the water-culture experiment, the seedlings raised from the seeds soaked in M/400 concentration of zinc sulphate solution were set up, on December12, 1958, in Pyrex beakers of 2-litre capacity, containing nutrient solution (Hoagland) deficient in zinc. In the control, the plants were raised from the seeds soaked in distilled water; these seedlings were raised separately in culture with and without zinc. Each treatment contained four plants. The salts used for seed soaking were laboratory reagents and those used in the nutrient solution were purified by

dithizone (Stout and Arnon, 1939). The water distilled from a Pyrex glass-still and tested by dithizone for its freedom from all metals, was used. The plants were harvested on February 16, 1959, after recording the number of tillers and fresh weights of leaves, stem and roots per plant.

Field experiment: Seeds of variety N.P. 718 were soaked separately in solutions of zinc sulphate (100 ppm. concentration of zinc), ferrous sulphate (100 ppm. concentration of iron) and boric acid (100 ppm. concentration of boron), with a ratio of 1:10 between the weight of the seed and the volume of each solution, for 18 hours at room temperature on October 16, 1959. These seeds, after rinsing with water, were sown in the field on October 17, 1959, adopting a simple randomized layout with four replications. In one of the controls the seeds were soaked in water, while in the other unsoaked seeds were used. The net plot size was 40 ft. × 18 ft. Ammonium sulphate at the rate of 20 lb. nitrogen per acre was applied to all the treatments about two months after sowing. In the field experiment, besides zinc two other salts —boron and iron— were also included, but their effects will not be discussed in this paper.

EXPERIMENTAL RESULTS

Zinc content: The zinc content of the seedling and endosperm of the seeds soaked in various concentrations of the zinc solution (M/100 to M/1000), was found to be much higher than that of the unsoaked seeds. The concentration of zinc absorbed in the seedlings and endosperm decreased with the decrease in the concentration of the soaking solution. The zinc concentration was found to be higher in the seedlings as compared to the endosperm (Table I).

It was interesting to note that higher concentration of zinc solution depressed the germination, whereas the lower concentrations had some favourable effect.

TABLE I. ABSORPTION OF ZINC FROM 200 M.L. OF ZINC SULPHATE SOLUTION IN 24 HOURS AT ROOM TEMPERATURE BY 20 GM. OF WHEAT SEEDS, TESTA AND ENDOSPERM

Concentration of zinc sulphate	Quantity of zinc absorbed (mg.)	Zinc as ppm. of dry weight		Percentage germination
		Seedlings	Endosperm	
Control (water-soaked)	Nil	26	24	55
M/100	34·8	715	360	40
M/200	26·9	645	315	60
M/400	14·7	475	315	73
M/600	11·6	465	264	83
M/800	9·4	330	195	87
M/1000	6·7	255	261	92

Water-culture experiment: From day-to-day observations on plants during the early stage of growth it was observed that out of the three series only the plants under control, which were maintained in the nutrient solution deficient in zinc, showed stunted growth, whereas those under the other two series (soaked in zinc and the control in the normal nutrient solution) had normal growth. The typical symptoms of zinc deficiency were noticed on the leaves of these plants (control without zinc) within three weeks. When the plants were about six weeks old, the growth of the plants from zinc-soaked seeds also decreased, and the symptoms of zinc deficiency appeared on the leaves. No ears emerged in the plants under control without zinc; in the plants from the zinc-soaked seeds, about two ears per plant emerged, but no grains were formed in them. In the plants under control with zinc, ear formation and grain filling was normal.

TABLE II. TOTAL NUMBER OF TILLERS AND FRESH WEIGHTS OF LEAVES, STEM AND ROOTS (PER PLANT)

Treatment	Number of tillers per plant	Fresh weight (gm.)			Total fresh weight (gm.)
		Leaves	Stem	Roots	
Control (+ Zn.)	21·5	31·6	75·1	28·2	134·9
Control (— Zn.)	11·0	8·4	12·8	11·7	32·9
Zinc-soaked (—Zn.)	15·7	11·9	16·1	13·4	41·4

The number of tillers and fresh weights of leaves, stem and roots of the plants from the zinc-soaked seeds and those under control without zinc were much reduced as compared to plants under control in the normal nutrient solution. But the reduction was far less in the plants from the zinc-soaked seeds than those under the control without zinc.

Field experiment: The data on the grain yield are given in Table III. The differences in the grain yield per acre between the treatment of soaking in zinc and the two controls were not statistically significant. Lodging occurred in the crop and the differences in the yield might probably be attributed to that.

TABLE III. MEAN YIELD OF GRAIN IN KGM. PER ACRE

Treatment	Yield in kgm. per acre	Statistical significance
Control (unsoaked)	1,671	. .
Control (water-soaked)	1,456	Not significant
Soaked in zinc sulphate	1,529	Not significant

S.Em. = ± 86

CONCLUSION

The zinc content of the seedlings and endosperm of wheat seeds was increased by soaking the seeds in zinc sulphate solution. The work of Roberts (1948) on cereals also showed that relatively larger quantities of mineral nutrients can be introduced into the seeds of cereals by soaking them in solutions containing the minerals. Similar observation was also made by Singh (*loc. cit.*) on cotton seeds. The major proportion of the zinc absorbed by the seeds from the zinc solution was located in the seedlings. The quantity of zinc absorbed decreased with the decrease in the concentration of the soaking solution.

Plants from the seeds soaked in zinc sulphate solution under nutrient solution deficient in zinc produced larger number of tillers and higher fresh weight of leaves, stem and roots than those soaked in distilled water only under nutrient solution without zinc; the former were, however, inferior in tiller number and fresh weights of plants to those from the controlled seeds grown in culture solution with zinc. The extra amount of zinc which was absorbed by the seeds from zinc sulphate solution during soaking helped the plants in making some growth, but was not enough to fulfil the requirement of the plant for zinc for the entire life cycle if it were raised in a nutrient solution deficient in zinc. These results are in agreement with the earlier results reported by the Singh (*loc. cit.*) on cotton seeds, and those of Roberts (*loc. cit.*) who suggested, in case of oats, that a large part of the manganese requirement, at least in the early stages of growth, could be provided by soaking; any further requirement could be made good by spraying.

The result of field trial indicated that the treatment of soaking wheat seeds in zinc sulphate solution, before sowing, had no influence on the grain yield. Similar observation was made (Singh, *loc. cit.*) in case of cotton. However, the soil in which the trial was conducted was not known to be deficient in zinc.

SUMMARY

The amount of zinc absorbed by wheat seeds (var. N.P. 718) from zinc sulphate solution of various concentrations, was determined in the seedlings and endosperm by the polorographic method.

The zinc content of both seedling and endosperm increased over that of control (water-soaked). The concentration of zinc in the seedlings was found to be higher than that in the endosperm, and the zinc content both in the seedlings and endosperm decreased with the decrease in the concentration of the soaking solution.

Under water-culture deficient in zinc, plants from zinc-soaked seeds showed better growth than those from water-soaked seeds for some time and developed symptoms of zinc deficiency later, thus indicating that the zinc absorbed by the seeds was not sufficient to meet the zinc requirement for the entire life-cycle of the plant.

Soaking of seeds of wheat in zinc sulphate solution had no influence on the yield of grain in the field which was not known to be deficient in zinc.

ACKNOWLEDGEMENTS

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EFFICIENCY OF DICALCIUM PHOSPHATE AS A FERTILIZER IN THE SOIL OF DELHI

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In recent years, much work has been done on the development of methods for the production of new phosphatic materials suitable for use as fertilizers. Dicalcium phosphate offers considerable scope and promise as a source of phosphorus for plant growth because of its high P_2O_5 content and consequent economy in handling and transportation costs. It is a chemically processed carrier of phosphorus with excellent physical condition, and can be kept for any length of time without reversion to unavailable forms. It is prepared by the action of hydrochloric acid on phosphate rock. The reaction can be represented as follows:

(i) $Ca_3\ (PO_4)_2 + HCl = Ca\ (H_2PO_4)_2 + 2\ CaCl_2$

(ii) $Ca\ (H_2PO_4)_2 + CaO = 2\ CaHPO_4 + H_2O$

The total P_2O_5 in the freshly prepared product is 36–41 per cent, out of which 32–34 per cent is soluble in neutral ammonium citrate and, as such, less liable to fixation in acidic and alkaline soils. It has a pH of 6·0.

Experiments to test the efficiency of various phosphatic fertilizers were carried out by Gilbert and Pember (1936) who got fairly comparable crop yields with either monocalcium phosphate, dicalcium phosphate or double superphosphate on sandy loam soils of the Agricultural Experiment Station, Rhode Island. Green (1938) found dicalcium phosphate to give very poor response as compared to water-soluble superphosphate on cereal crops in phosphorus-deficient alkaline soils, while Mooers (1929) found citrate-soluble dicalcium phosphate to be as good as water-soluble superphosphate. He found that field trials with corn, wheat, millets, cow-peas, soyabeans and potatoes in a number of phosphate-deficient soils in Tennessee showed no significant differences in their effect upon crop yield.

In the U.S.A., Allison *et al.* (1941), Conner and Adams (1926), Haskins (1921, 1922), Mooers (*loc. cit.*), Ross *et al.* (1932) and Thronton (1932), and in Europe Drevspring *et al.* (1929) and Wlilhelmj *et al.* (1929) showed that citrate-soluble phosphates, i.e., dicalcium and alkali phosphates, were usually excellent sources of phosphorus for plant growth.

Stanford and Nelson (1949) studied the response of oats to sources of phosphorus at three locations. Significant response to phosphorus was noticed only on the Webster silty clay-loam at pH 6·0. At this location, dicalcium phosphate produced as much response as did superphosphate. By the use of tagged superphosphate, they found that the precentage of plant phosphorus derived from dicalcium phosphate was much lower than that derived from superphosphate; but Jacob and Rose (1940) showed dicalcium phosphate to be more efficient source of P_2O_5 than superphosphate.

In India, Idnani *et al.* (1955) reported dicalcium phosphate to be superior to superphosphate, bone-meal and farmyard manure. Later, in T.C.M. trials on wheat (I.C.A.R., 1959) at Nawanshahr, Bhadson, Alipur, Pisangunj, and Raisingnagar, dicalcium phosphate was reported to be slightly inferior to superphosphate, while on acidic soils of Chalakudy (Kerala) having a pH range of 5–6, dicalcium phosphate was found to be superior to monocalcium phosphate.

Information regarding the efficiency and behaviour of dicalcium phosphate on the neutral and alkaline soils of Delhi State is lacking. The present experiment was conducted to find out the comparative efficiency of this fertilizer on wheat and maize.

EXPERIMENTAL

A field experiment was conducted on the Institute Farm at New Delhi from *rabi* 1957-58 to *kharif* 1959. The soil of the field is sandy-loam having the following mechanical and chemical composition in the top (0–9 in.) of the soil profile.

pH	7·2
Coarse sand	0·86 per cent
Fine sand	71·02 per cent
Silt	13·41 per cent
Clay	13·30 per cent
CaO	2·39 per cent
P_2O_5	0·21 per cent
K_2O	2·87 per cent
Nitrogen	0·07 per cent

The experiment was laid out in randomized blocks with six replications on plots of 30 ft. × 24 ft.

The treatments included are given below:

A	No manure (control)
B	Ammonium sulphate nitrate at 40 lb. N per acre.
C	Nitrogen as in 'B' + superphosphate (16 per cent P_2O_5) at 80 lb. of P_2O_5 per acre.
D	Nitrogen as in 'B' + dicalcium phosphate (34 per cent P_2O_5) at 80 lb. of P_2O_5 per acre.

The fertilizers were broadcast before sowing each crop and worked into the soil by ploughing. Wheat (N.P. 710) was grown in *rabi* and maize (N.P. 2—yellow) in the *kharif* seasons under irrigated conditions.

Samples of grain and straw of wheat and grain and stover of maize were taken from each of the 24 plots and the composite samples of each treatment were analyzed for nitrogen and phosphoric acid.

Nitrogen was determined by the Kjeldahl method (A.O.A.C., 1955) and P_2O_5 by Koening and Jhonson's method (1942).

RESULTS AND DISCUSSION

The yield figures obtained for wheat and maize during two years are given in Table I. The maize yield obtained in 1958 was low because of late arrival of fertilizers and consequent delay in sowing the crop.

TABLE I. YIELD OF WHEAT AND MAIZE GRAIN IN MD. PER ACRE

Treatment per acre	Wheat			Maize		
	1957–58	1958–59	Average	1958	1959	Average
A. No manure (control)	8·9	9·6	9·3	4·1	10·2	7·2
B. N	14·1	15·7	14·9	6·6	18·7	12·6
C. NP (super)	19·3	21·7	20·5	7·9	17·0	12·5
D. NP (dicalcium phosphate)	18·7	17·0	17·9	7·3	16·6	12·0
'F' test	Sig.**	Sig.**		Sig.**	Sig.*	
S.Em.	±0·89	±1·52		±0·29	±1·88	
C.D. at 5 per cent	2·69	4·58		0·89	5·84	

*and **indicate significance at 5 per cent and 1 per cent levels respectively.

Both the crops responded significantly to all the fertilizer treatments as compared to the control (no manure). The effect of superphosphate was found to be slightly better than that of dicalcium phosphate, and this difference was significant on the second crop of wheat. Further, the response due to superphosphate was found to be significant on all crops, except the last crop of maize, while with dicalcium phosphate only the first crop of wheat showed significant difference. Table II gives the total uptake of nitrogen and P_2O_5 by wheat and maize crops for all the seasons.

TABLE II. TOTAL UPTAKE OF NITROGEN AND P_2O_5 BY WHEAT AND MAIZE IN LB. PER ACRE

Treatment per acre	Wheat						Maize					
	1957–58		1958–59		Average		1958		1959		Average	
	N	P_2O_5	N	P_2O_5	N	P_2O_5	N	P_2O_5	N	P_2O_5	N	P_2O_5
A. No manure	29·60	14·12	19·89	8·66	23·25	11·49	14·78	11·38	26·73	18·42	20·76	14·90
B. N	41·90	24·06	32·81	11·73	37·36	17·90	18·75	15·49	50·13	26·35	34·44	20·92
C. NP	57·68	33·61	46·63	20·56	52·16	27·19	25·79	20·92	43·67	33·92	34·73	27·42
D. NP (dicalcium phosphate)	55·38	31·34	37·63	17·48	46·51	24·41	22·36	18·61	42·61	29·96	32·49	24·29

Marked increases in the total uptake of N and P_2O_5 by both the crops were observed throughout under all the fertilizer treatments. The uptake of nitrogen in general, and that of P_2O_5 in particular, increased under the treatments C and D (NP) as compared to the treatment B (nitrogen alone). The difference between the uptake of N and P_2O_5 under the treatments C (NP:P as super) and D (NP:P as dicalcium phosphate) is not appreciable, the former showing a little superiority over the latter.

SUMMARY

Results of field trials conducted with superphosphate (containing 16 per cent water-soluble P_2O_5) and dicalcium phosphate (containing 34 per cent citrate-soluble P_2O_5) in the neutral soils of Delhi showed that superphosphate shows a trend of slight superiority over dicalcium phosphate.

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INFLUENCE OF AMMONIUM SALTS ON THE ADSORPTIVE BEHAVIOUR OF INDIAN MUSCOVITE WITH PHOSPHORIC ACID

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Appreciable quantities of mica have been observed in Indian soils. Jeffries and Anthony (1948) recorded that the average mica content of the sediment of the earth is about 20 per cent. It is natural to conjecture that the physico-chemical changes brought about by the addition of certain fertilizers to micaceous soils would be considerably directed by the amount and kind of mica present in such systems. In the present study, an attempt has been made to evaluate the extent of phosphate removal from phosphoric acid by a mica, viz., muscovite, in presence of three ammonium salts commonly used as fertilizers and also to probe into the mechanism of intake under such conditions. Previously, Mitra and Dharam Prakash (1955) studied the phosphate intake capacity of this sample of muscovite without adding any ammonium salt to the reacting systems.

The sample of muscovite was obtained from the collection of late Dr. S. P. Mitra. It was identified by him by X-ray analysis. The chemical analysis of the mineral was done according to the method outlined by Washington (1930).

EXPERIMENTAL

The muscovite mineral was first finely powdered and then passed through a 100-mesh sieve. The well-pulverized sample was then stocked in a bottle for the adsorption experiments.

In several 250-ml. flasks containing 2·0 gm. mineral, different amounts of ammonium salts (B. D. H., Analar) and 100 ml. of phosphoric acid containing 51·9738 mg. of P_2O_5 were added. The mixtures were occasionally shaken. The flasks were afterwards kept in a thermostat for 24 hours at 32°C. By taking suitable aliquot portions, the supernatant liquid was carefully analyzed for P_2O_5 content by the usual ammonium phosphomolybdate method. The aluminium content of the supernatant liquid was estimated volumetrically by means of 8-hydroxyquinoline, as described by Vogel (1953).

Release of aluminium was also studied in the absence of phosphoric acid, using solutions of the ammonium salts of the same concentration as before. The pH of the solutions was adjusted by HCl or NH_4OH according to the final pH observed in the respective adsorption experiments.

All pH measurements were carried out by Leeds Northrup pH meter. A glass-colomel electrode system supplied by the same manufacturer was employed. Before measurements, the calibration of the scale was made with the help of a pthallate buffer which was checked occasionally.

The adsorption of phosphates reported here are in terms of mg. of P_2O_5 in 100 ml. solution.

Chemical analysis of Indian muscovite (*per cent*)

(Oven-dry basis)

SiO_2	47·223
Al_2O_3	39·562
Fe_2O_3	0·254
P_2O_5	..
K_2O	12·822
MgO	0·114
CaO	0·025

TABLE I. VARIATIONS IN pH OF H_3PO_4 WITH THE ADDITION OF DIFFERENT AMOUNTS OF AMMONIUM SALTS

Solution	pH		
	NH_4Cl	NH_4NO_3	$(NH_4)_2SO_4$
H_3PO_4 + 0·5 gm. NH_4 salt	3·20	3·60	3·70
H_3PO_4 + 1·0 gm. NH_4 salt	3·50	3·85	3·92
H_3PO_4 + 1·5 gm. NH_4 salt	3·85	4·01	4·30
H_3PO_4 + 2·0 gm. NH_4 salt	4·40	4·30	4·45

TABLE II. ADSORPTION OF PHOSPHATE FROM H_3PO_4 BY INDIAN MUSCOVITE

Initial concentration mg.	Adsorption mg.	Release of aluminium with water (pH 3·25) (mg.)	Initial pH	pH of the mixture	Aluminium released (mg.)
51·9738	4·32	24·82	3·1	3·25	68·86

TABLE III. ADSORPTION OF PHOSPHATE FROM H_3PO_4 BY INDIAN MUSCOVITE IN PRESENCE OF NH_4Cl

Reacting mixture	Adsorption (mg.)	pH of the mixture	Aluminium released (mg.)
2·0 gm. mineral + H_3PO_4 + 0·5 gm. NH_4Cl	5·25	3·60	62·02
2·0 gm. mineral + H_3PO_4 + 1·0 gm. NH_4Cl	9·08	3·80	52·42
2·0 gm. mineral + H_3PO_4 + 1·5 gm. NH_4Cl	18·72	3·95	25·82
2·0 gm. mineral + H_3PO_4 + 2·0 gm. NH_4Cl	10·50	4·60	66·25

TABLE IV. ADSORPTION OF PHOSPHATE FROM H_3PO_4 BY INDIAN MUSCOVITE IN PRESENCE OF NH_4NO_3

Reacting mixture	Adsorption (mg.)	pH of the mixture	Aluminium released (mg.)
2·0 gm. mineral + H_3PO_4 + 0·5 gm. NH_4NO_3	4·43	3·70	55·32
2·0 gm. mineral + H_3PO_4 + 1·0 gm. NH_4NO_3	9·04	3·85	43·02
2·0 gm. mineral + H_3PO_4 + 1·5 gm. NH_4NO_3	9·82	4·10	47·28
2·0 gm. mineral + H_3PO_4 + 2·0 gm. NH_4NO_3	6·24	4·20	72·97

TABLE V. ADSORPTION OF PHOSPHATE FROM H_3PO_4 BY INDIAN MUSCOVITE IN PRESENCE OF $(NH_4)_2SO_4$

Reacting mixture	Adsorption (mg.)	pH of the mixture	Aluminium released (mg.)
2·0 gm. mineral + H_3PO_4 + 0·5 gm. $(NH_4)_2SO_4$	0·21	3·85	64·02
2·0 gm. mineral + H_3PO_4 + 1·0 gm. $(NH_4)_2SO_4$	0·71	4·01	70·56
2·0 gm. mineral + H_3PO_4 + 1·5 gm. $(NH_4)_2SO_4$	0·46	4·35	81·64
2·0 gm. mineral + H_3PO_4 + 2·0 gm. $(NH_4)_2SO_4$	0·30	4·50	90·10

TABLE VI. RELEASE OF ALUMINIUM FROM INDIAN MUSCOVITE BY SOLUTIONS OF NH_4Cl, NH_4NO_3 AND $(NH_4)_2SO_4$ IN ABSENCE OF PHOSPHORIC ACID

Quantity of salts used in solutions	pH of the solutions			Aluminium released (mg.)		
	NH_4Cl	NH_4NO_3	$(NH_4)_2SO_4$	NH_4Cl	NH_4NO_3	$(NH_4)_2SO_4$
0·5	3·60	3·70	3·85	80·05	71·35	64·84
1·0	3·80	3·85	4·01	88·22	79·42	73·37
1·5	3·95	4·10	4·35	97·35	87·85	82·92
2·0	4·60	4·20	4·50	106·42	96·82	91·34

RESULTS AND DISCUSSION

The adsorption of phosphate from phosphoric acid by Indian muscovite was found to increase in presence of ammonium chloride and ammonium nitrate salts. The extent of phosphate intake by the clay mineral sample, however, decreased in presence of ammonium sulphate. With 1·5 gm. ammonium chloride and ammonium nitrate, maximum adsorption was observed. With ammonium sulphate, maximum adsorption was noted at 1·0 gm. concentration of the salt. In every case, adsorption

was accompanied by an increase in the resultant pH. Appreciable release of aluminium was also found from the muscovite sample. The release of aluminium was less in the presence of phosphoric acid.

Practically in all cases, maximum adsorption occurred wherever the final pH of the reacting system approached 4. There was considerable decrease in the intake as the reaction shifted farther from this value both ways. Another interesting fact is that there is an appreciable fall in the aluminium concentration at the maximum point of phosphate adsorption. It seems that at pH 4 the association of released aluminium and phosphate was favoured, leading to the precipitation of insoluble aluminium phosphate. It is interesting to note that in all cases, approximately one part of P_2O_5 combined with four parts of the released aluminium (compare Tables III, IV and V with Table VI). Thus, it appears that in the precipitated aluminium phosphate, the ratio of $Al:P_2O_5$ is about 4. It may be of interest to recall here the observations of Mattson (1953) that " phosphate adsorption (precipitation) due to aluminium begins at a high pH but attains a maximum " at pH 4 " which, in their hydroxyl, silicate and humate combinations, are here actively displaced by H ions. " The mineral muscovite is a potassium alumino-silicate $[(OH)_4\ K_2\ (Si_6.Al_2)\ Al_4\ O_{20}]$ containing about 38·5 per cent Al_2O_3 (29·562 per cent in our sample) and it is, therefore, likely that some such precipitation reaction has occurred in the systems investigated. Together with H^+ cations, the added NH_4^+ ions also seem to replace Al^{+++} from the mineral, thus facilitating increased phosphate precipitation and final pH.

In the reacting systems containing ammonium sulphate, it is interesting to note that very little of the total released aluminium has been utilized for association with phosphate. It is yet to be seen how SO_4^{2} ions interfere with the precipitation of aluminium phosphate which does not seem to occur in presence of NO_3^- and Cl^- ions.

SUMMARY

Adsorption of phosphate from phosphoric acid by a sample of Indian muscovite was carried out in presence of three ammonium salts, viz., ammonium chloride, ammonium nitrate and ammonium sulphate. The adsorption increased with the addition of ammonium chloride and ammonium nitrate, whereas ammonium sulphate depressed the process. In all cases, the adsorption was mainly due to the precipitation of insoluble aluminium phosphate in which the ratio of $Al:P_2O_5$ was found to be about 4. So_4^{2-} appeared to reduce the association of phosphate ion with aluminium. No such depreciating effect was observed with NO_3^- and Cl^- ions.

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AN IMPROVED TECHNIQUE FOR METHYL BROMIDE FUMIGATION

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Methyl bromide, because of its gaseous nature at ordinary temperatures, is more difficult to use than some of the other liquid fumigants. This fumigant is generally available in pressurized cylinders, and its application from these cylinders involves the use of some sort of dispenser. The laboratory dispenser described by Lubatti and Smith (1948) has to be maintained at a very low temperature of—30°C while it is being filled from the pressurized cylinder; alternatively, sealed glass-ampoules containing liquefied gaseous fumigant have to be used. An attempt was made by Sen Gupta (1951), who adopted the technique described by Dudley *et al.* (1940) with some modifications to apply gaseous methyl bromide to a fumigatorium after collecting it from the pressurized cylinder in a suitable container. The principle involved in this technique is the collection of methyl bromide by displacement of water and then forcing a measured quantity of the fumigant by exerting water-pressure. This method may be suitable for introducing the gas in a big fumigation chamber (7·9 cft. used by him) where a small error in measuring the gas may not materially affect the concentration of the fumigant; but such an error will alter appreciably the concentration of the fumigant in a small fumigatorium. The present technique is based on the same principle as that used by Sen Gupta, but with a modification that ensures an accurate measurement and application of even the smallest quantities of the gaseous fumigant.

TECHNIQUE

The technique comprises of three steps: (a) collection of the gas in an aspirator bottle, (b) collection of the gas in measuring tube, and (c) application of the gas into a fumigation chamber.

Collection of the gas in aspirator bottle (*Fig.* 1)*:* On opening valves Y^1 and Y^2 of the cylinder C containing liquid methyl bromide under pressure (available from the National Fire Protection Co. Ltd., Surrey, England), gaseous methyl bromide is collected in an aspirator bottle (A_1), previously filled with water. The incoming fumigant forces the water out through the outlet X^1. In this process, it is very important to manipulate carefully the valves of the cylinder to avoid the bursting of the aspirator bottle due to the sudden rush of the gas. When sufficient volume of the gas is collected, the aspirator bottle is disconnected from the cylinder.

Collection of the gas in measuring tube (*Fig.* 2)*:* The aspirator bottle A_1 is connected on one side to another aspirator bottle A_2, also full of water, and on the other to a water-filled pyrex glass measuring tube T, of 6·25 cm. diameter and 60 cm. length,

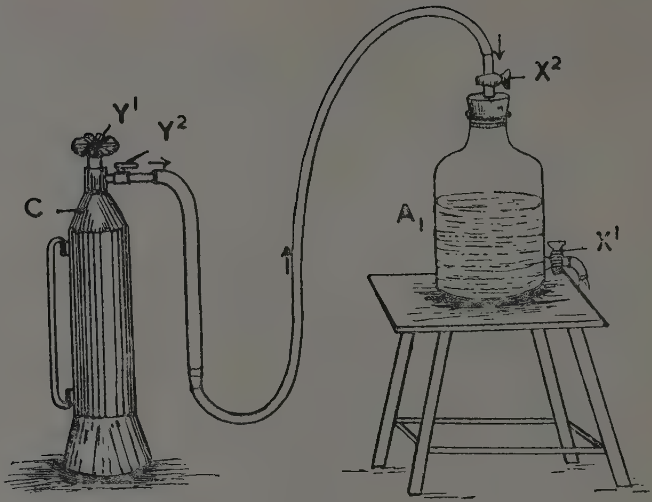

FIG. 1.

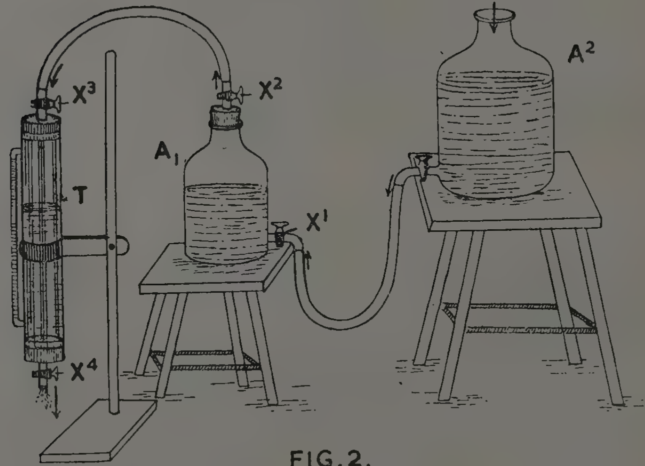

FIG. 2.

and having a graduated side-arm. A 10-ml. microburette, after removing its stop-cock, is fitted to the tube and serves as the graduated side-arm. After making the connections, the water-pressure that is applied from the aspirator bottle A_2 to the aspirator bottle A_1 forces the fumigant into the measuring tube T, and the displaced water runs out through an outlet X^4. When sufficient gas is collected in the measuring

tube, the latter is cut off from the aspirator bottle by manipulating the stop-cocks X^2, X^3 and X^4.

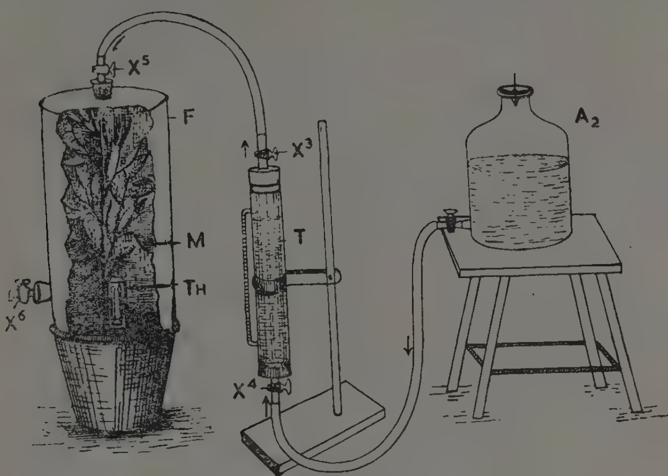

FIG. 3.

Application of the gas into fumigatorium (*Fig.* 3): The measuring tube T, containing gaseous methyl bromide, is connected on one side to the aspirator bottle A_2 filled with water, and on the other to the fumigation chamber F of 41·407-litre capacity (described by Pradhan *et al.*, in press), used for the fumigation of the potted plants. By applying water-pressure from the aspirator bottle A_2, the desired volume of the fumigant is forced into the fumigation chamber. The volume of the fumigant introduced can be calculated, since one ml. of the side-arm was found to be equal to a total displacement of 50 ml. from the measuring tube T. With such an apparatus, volumes of gaseous fumigant varying from 25—500 ml. can be applied with a high degree of accuracy. If more than 500 ml. of the fumigant is required, the measuring tube T may be refilled and the procedure repeated.

For the calculation of the exact weight of the gaseous fumigant, the effect of temperature and fluctuations in the atmospheric pressure have to be taken into consideration (Boyle's and Charles's Laws). Therefore, using the relationship, " A gram-mole of any gas at N.T.P. measures 22·414 litres, " the weight of one litre of methyl bromide at different temperatures from 20° to 35°C—a range which covers the temperatures at which fumigation is generally carried out—at normal atmospheric pressure and at the average pressures from October to April and May to September at New Delhi, has been calculated and presented in Table I, with a view to simplify the calculation of the dosage at any required temperature. Similarly, a correlation for the fluctuations in the atmospheric pressure may be made according to the local pressure conditions where the fumigation has to be carried out.

TABLE I. WEIGHT OF ONE LITRE OF GASEOUS METHYL BROMIDE IN GM. AT DIFFERENT TEMPERATURES AND ATMOSPHERIC PRESSURES

Temperature °C	Normal pressure 760 mm. of Hg	Average pressure at New Delhi	
		For months of October to April 752·4 mm. of Hg	For months of May to September 744·1 mm. of Hg
20	3·9470	3·9075	3·8641
21	3·9336	3·8943	3·8510
22	3·9203	3·8811	3·8380
23	3·9070	3·8680	3·8250
24	3·8939	3·8550	3·8121
25	3·8808	3·8420	3·7993
26	3·8678	3·8291	3·7866
27	3·8549	3·8164	3·7739
28	3·8421	3·8037	3·7614
29	3·8294	3·7911	3·7490
30	3·8167	3·7785	3·7366
31	3·8042	3·7662	3·7243
32	3·7917	3·7538	3·7121
33	3·7793	3·7415	3·6999
34	3·7670	3·7293	3·6879
35	3·7548	3·7173	3·6759

Tests were carried out to evaluate the accuracy of the technique. A concentration of 27·3 mg. per litre was obtained in the fumigation chamber F. About 10 minutes thereafter, a sample of one litre was drawn from the outlet X^6 and the quantity of methyl bromide present was determined after Lewis (1945), using sodium methylate and silver nitrate. The estimation was repeated thrice. It was found that the difference between the dosage applied and the concentration chemically determined was $\pm 0{\cdot}22$ per cent.

SUMMARY

Gaseous methyl bromide is collected in an aspirator bottle from pressurized cylinder. It is then forced under water-pressure into a tube fitted with a graduated side-arm from where accurately measured volume of the fumigant is introduced into the fumigation chamber. The calculation of the exact weight of one litre of

gaseous methyl bromide has been done at temperatures ranging from 20° to 35°C and at an average atmospheric pressure of 744·1 mm. of mercury (the average pressure for the months May to September) and 752·4 mm. of mercury (the average pressure for the months of October to April at New Delhi), with a view to render the subsequent calculation of the dosage easier.

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INTERRELATIONSHIP BETWEEN ELECTRICAL CONDUCTIVITY OF 1:5, AND SATURATION EXTRACTS AND TOTAL SOLUBLE SALTS IN SALINE-ALKALI SOILS OF THE GANGETIC ALLUVIUM IN UTTAR PRADESH

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In a series of earlier publications from these laboratories the characteristics, diagnostic techniques and methods of salinity appraisal including a salinity crop rating scale for saline-alkali soils of Indian Gangetic alluvium have been reported (Agarwal and Yadav, 1954, 1956a, 1956b). The present investigations cover the study of interrelationships between the modern laboratory determinations for salinity appraisal and the older indices which were used in earlier days, and were based on determination of salts in 1:5 water extracts. Such a study would facilitate comparison of the older data with the modern indices for the salinity appraisal, which are based on the analysis of saturation extract of soil.

Work in this branch has been reported from the U.S. Salinity Laboratory by Richards (1954), who has worked out a relationship between the total soluble salt contents and the electrical conductivity of the saturation extracts of soils, and has derived the following equation:

$$Pss = \frac{Psw \times Pw}{100}$$

where Pss is the percentage salt in soil, Psw is the percentage of salt in water and Pw is the percentage of water in soil. Psw, on the other hand, is equal to $EC \times 10^3 \times 0{\cdot}064$. This relationship was to a large extent dependent on the saturation percentage of the soil. Magistad *et al.* (1945) deduced a multiplication factor of 10 for converting the specific conductance values into the salt concentration of soil solutions expressed in m.e. per litre or equivalent parts per million parts (e.p.m.) of the solution. Piper (1950) similarly suggested the working out of the total soluble salt contents by multiplying the specific conductance of 1:5 soil water suspension with an emperical factor of 375. Hoon, Malhotra and Jain (1941), on the other hand, obtained relationship of $10^3S = 0{\cdot}314C + 25{\cdot}4$ between total soluble salt contents (S) obtained by gravimetric measurements and conductivities of soil suspensions (C) for soil having a range of conductivity not exceeding 400. Yet another relationship has been proposed by Jackson (1949), where the salt concentration of soil solution in parts per million is obtained by multiplying the values of specific conductance in mmhos/cm. with an emperical figure of 700.

The objective of the current investigations could be achieved through the examination of soils from saline-alkali areas of the Indian Gangetic alluvium both by the old as well as the modern techniques and by working out the various interrelationships between the values. The effect of soil heterogeneity and variations in soil

characters could be minimized by increasing the number of soil samples collected from widely divergent tracts, such that the studies could embrace both the more prevalent carbonate-bicarbonate soils and the less common sulphate-chloride soils.

MATERIAL AND METHODS

Surface-soil samples from widely divergent saline-alkali tracts of Uttar Pradesh, covering a wide range of soil salinity, were collected for these investigations. In all, 60 samples typical of the different categories of saline-alkali soils including both the carbonate-bicarbonate and the sulphate-chloride predominating types—almost all of them being calcareous in nature—were collected from *usar* areas of the mid-eastern *doab* of the state. Each of these soil samples was subjected to detailed examination both for the saturation as well as 1:5 soil-water extract. Saturation extracts were prepared by the method indicated by Richards (*loc. cit.*) filtering the paste on a modified Houston pump under pressure. The soil-water extracts were obtained by shaking the soil with distilled water in 1:5 soil-water ratio for one hour. The paste was filtered on the Houston pump and the extract used for examinations. Each of the extracts was tested on Solu Bridge for electrical conductivity measurement which was expressed in mmhos/cm. The pH determinations were made on a Beckman H-2 model pH-meter. Total dissolved salts were estimated in 1:5 extract by evaporating a known volume of extract on a water-bath followed by drying in an air-oven at 105°C to a constant weight, and the results expressed in gm. per 100 gm. of soil. Determinations of pH were made on 1:2·5 soil-water paste to obtain the pH values conforming to the values usually obtained in the routine laboratory examination of soils.

The saturation extracts as well as 1:5 soil-water extracts were analyzed for all the anions, viz., CO_3, HCO_3, Cl, SO_4 and NO_3, by the usual volumetric or gravimetric methods. Bivalent cations were measured volumetrically by Versenate method and values of monovalent cations worked out by subtracting the bivalent values from the total values of anions obtained by summing up of the contents of individual anions. The contents of anions and cations were expressed in milli-equivalents per litre.

EXPERIMENTAL

The analytical data in respect of saturation percentage, pH, electrical conductivity of different extracts, and total dissolved solids are presented in Table I. The ratios between the percentage of total dissolved salts in 1:5 soil-water extracts and the electrical conductivity of 1:5 soil-water extracts were calculated by dividing the values separately and are presented alongside the other analytical data in Table I. The total ionic concentration obtained by summing up the values of individual anions of saturation extracts, measured separately but not shown in the paper, have also been indicated in Table I. The relationship between total soluble salts and electrical conductivity of 1:5 extract has been graphically represented in Fig. 1.

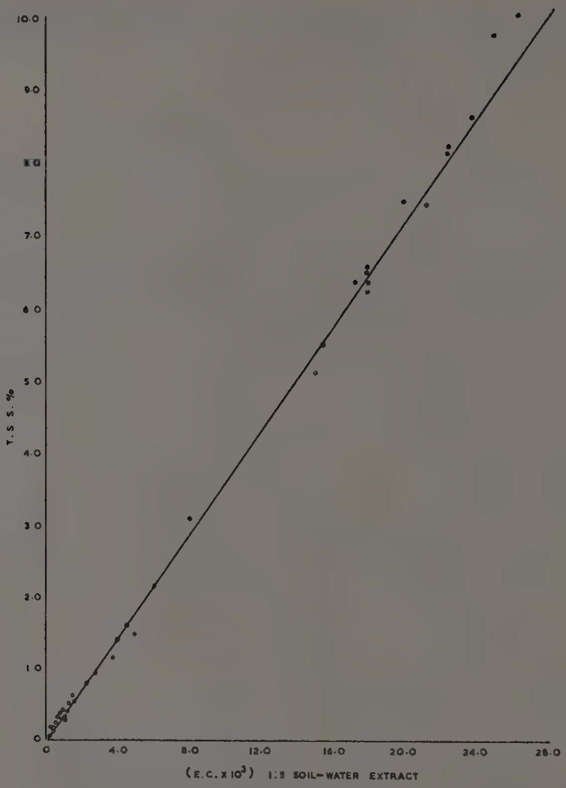

FIG. 1. RELATIONSHIP BETWEEN PERCENTAGE OF TOTAL SOLUBLE SALTS AND ELECTRICAL CONDUCTIVITY OF 1:5 SOIL-WATER EXTRACT

RESULTS AND DISCUSSION

The data presented in Table I indicate that the soils under investigation cover a very wide range of textures, as is evident from the values of saturation percentage, which ranged between 27 per cent and 54 per cent, though the greater accumulation of samples was between 30 per cent and 40 per cent. Comparatively heavier-textured soils, which are generally not very common at the surface, were few in number.

The pH values of saturation extracts, were in most of cases slightly lower than the values obtained on 1:5 soil-water extracts which in turn were more than the values on 1:2·5 soil-water paste. This type of behaviour has already been reported by Richards (*loc. cit.*) for American soils.

In electrical conductivity measurements, the values for saturation extracts, on the whole, were always more than those of 1:5 soil-water extracts, the range varying from 2·3 to 14·44 times. However, these differences could be narrowed down if the soils are compared on soil-group basis depending on their salinity status and consisting of high-salinity, sulphate-chloride type, high-salinity, carbonate-bicarbonate type and low-salinity groups of soils. The data can thus be separated out in the groups of 27 soils of high salt content (E.C. $\times 10^3$ being 12 to 300) and 33 soils of low salt contents with electrical conductivity 0·5—10 mmhos/cm. The analytical data of the anionic portion of saturation extract (not incorporated in this paper) however revealed that the first group of the high conductivity soils is predominatingly rich in sulphate-chloride ions, giving very high conductivity values ranging between 75 and 300 mmhos/cm. The second group of low conductivity contains soils rich in carbonate and bicarbonate ions.

Certain well-defined inter-relationships are observed in the analytical data, specially in respect of electrical conductivity of saturation extract and 1:5 soil-water extract along with their correlation in the total soluble salt contents of the soils. These relationships have been described below.

Relationship between electrical conductivity of 1:5 soil-water extract and saturation extract: The ratios between the electrical conductivity of saturation extract and that of 1:5 soil-water extract shown in column 7 indicate a fair degree of uniformity within the two salinity groups, the values varying from 9·8 to 13 in high-salinity sulphate-chloride groups, with maximum accumulation of samples at the value 12·2. The ratio of this interrelationship in the carbonate-bicarbonate type of high-salinity soils is, in general, somewhat less than in the preceding group of soils, the range varying from 6·6 to 14·44 with an overall average of 11·2 for this group of soil. Soils of low-salinity class have, in general, much lower ratio with broad range of variation between a maxima of 8·3 and a minima of 1·66. The overall average for 33 samples of this class is 4·95. The relationship has been graphically presented in Fig. 2, separately for the two salinity groups of soils which are both, more or less, linear.

Relationship between percentage of total soluble salts and electrical conductivity of 1:5 soil-water extract: The relationship between the percentage of total soluble salts and the electrical conductivity of 1:5 soil-water extract for 60 samples reported in column 10 of the table and graphically represented in Fig. 1, also show a fair degree of uniformity, the average ratio being 0·348. The relationship in the figures show a perfect fit straight-line correlation which can be safely said to be linear. In view of this rigid linear relationship, the values of total soluble salts in gm. per 100 gm. of soil can be safely deduced by the following equation.

$$\text{Percentage total soluble salts} = (\text{E.C.} \times 10^3)\ 1{:}5\ \text{extract} \times 0{\cdot}348.$$

Relationship between total ionic concentration and electrical conductivity of saturation extracts: The total ionic concentration computed from the determination of individual anions when compared with the electrical conductivity of saturation extract, as shown in column 11 of Table I, shows a ratio varying from 4·04 to 15·00, the average of all the 60 samples being 9·47. The broad variation in range warrant consideration of these values on the basis of soil groupings indicated earlier. A comparison made on these lines indicate

a better degree of uniformity in this relationship in two broadly different saline groups of soils, with high and low salinity status.

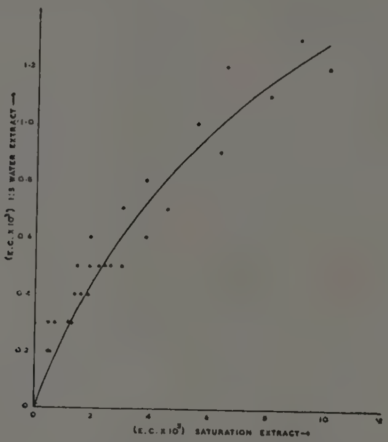

FIG. 2a. RELATIONSHIP BETWEEN ELECTRICAL CONDUCTIVITY OF 1:5 SOIL-WATER EXTRACT AND SATURATION EXTRACT IN LOW-SALINITY CO_3—HCO_3 GROUP

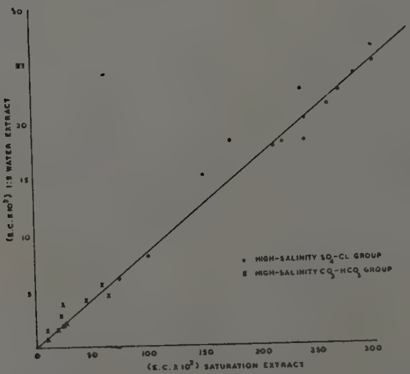

FIG. 2b. RELATIONSHIP BETWEEN ELECTRICAL CONDUCTIVITY OF 1:5 SOIL-WATER EXTRACT AND SATURATION EXTRACT IN HIGH-SALINITY CO_3—HCO_3 GROUP AND SO_4—CL GROUP

TABLE I. CHARACTERISTICS OF THE SOILS USED IN THE STUDY AND THEIR RELATIONSHIP BETWEEN TOTAL SOLUBLE SALTS AND ELECTRICAL CONDUCTIVITY

Saturation percentage	pH			E. Conductivity in millimhos/cm.		$\frac{(E.C. \times 10^3)\ S.E.}{(E.C. \times 10^3)\ 1{:}5\ Extract}$	T.S.S. %	Total soluble cations me/L	$\frac{T.S.S.\ \%}{(E.C. \times 10^3)\ 1{:}5\ extract}$	$\frac{T.S.C.}{(E.C. \times 10^3)\ S.E.}$
	1 : 5 Soil-water extract	1 : 2·5 Paste	Saturation extract	1 : 5 Soil water extract	Saturation extract					
Group: High-salinity sulphate-chloride type										
31	8·7	9·3	8·4	25·0	300·0	12·0	9·634	3622	0·385	12·1
32	7·8	8·9	7·8	26·3	300·0	11·4	9·924	3412	0·377	11·4
32	9·4	10·0	9·3	23·8	280·0	11·8	8·526	3209	0·358	11·5
31	8·9	9·7	9·2	22·5	270·0	12·0	8·023	3184	0·356	11·8
33	8·6	9·6	8·7	21·3	260·0	12·0	7·336	2943	0·344	11·3
34	9·1	9·6	9·0	20·0	240·0	12·0	7·372	2750	0·368	11·5
33	7·5	8·6	7·7	22·5	237·5	10·6	8·132	2648	0·361	11·2
33	9·5	10·0	9·4	18·0	220·0	12·2	6·404	2527	0·356	11·5
31	9·6	9·9	9·3	18·0	240·0	13·3	6·405	2527	0·356	10·5
32	8·7	9·4	8·6	18·0	220·0	12·2	6·284	2554	0·349	10·2
33	8·3	9·1	8·1	18·0	220·0	12·2	6·155	2381	0·342	10·8
34	9·5	9·9	9·3	17·5	212·5	12·1	6·282	2267	0·358	10·7
34	9·0	9·5	8·5	15·5	175·0	11·3	5·438	1973	0·351	11·2
38	7·7	8·2	7·5	18·0	175·0	9·7	6·392	1625	0·355	9·3
40	7·7	8·2	7·9	15·0	150·0	10·0	5·069	1287	0·338	8·6
44	10·0	10·3	9·9	8·0	100·0	12·5	3·040	1013	0·380	10·1
41	9·9	10·1	9·7	6·0	75·0	12·5	2·104	733	0·351	9·8
Group: High-salinity carbonate-bicarbonate type										
33	10·0	10·0	9·7	4·5	65·0	14·4	1·576	588	0·350	9·1
39	9·9	9·9	9·7	5·5	60·8	10·9	1·480	570	0·296	9·5
41	9·8	9·9	9·7	4·0	46·03	11·6	1·380	409	0·345	8·8
38	9·6	10·4	9·8	1·6	21·3	13·3	0·552	265	0·345	12·5
42	9·7	10·1	9·8	2·2	28·8	13·1	0·770	242	0·350	8·4
34	10·3	10·3	10·0	2·2	27·0	12·3	0·778	231	0·354	8·6

TABLE I—*Contd.*

Saturation percentage	pH			E. Conductivity in millimhos/cm.		(E.C.×10³) S.E. / (E.C.×10³) 1:5 Extract	T.S.S. %	Total soluble cations me/L	T.S.S. % / (E.C.×10³) 1:5 Extract	T.S.C. / (E.C.×10³) S.E.
	1:5 Soil-water extract	1 : 2.·5 Paste	Saturation extract	1:5 Soil:-water extract	Saturation extract					
38	9·7	10·2	9·8	3·8	25·0	6·6	1·134	710	0·298	8·4
36	9·9	10·0	9·8	2·8	23·8	8·5	0·934	197	0·334	8·3
35	9·2	9·6	9·6	0·9	12·0	13·3	0·300	145	0·333	12·1
34	9·8	10·3	9·8	1·5	12·0	8·0	0·512	132	0·341	12·0
Group: Low-salinity carbonate-bicarbonate type										
36	9·1	9·6	9·0	1·1	8·0	7·3	0·380	74	0·345	9·2
35	8·1	8·3	7·9	1·2	10·0	8·3	0·382	71	0·318	7·1
40	9·0	10·0	9·1	1·3	9·0	6·9	0·490	70	0·377	7·8
38	9·2	10·1	9·2	0·9	8·8	9·8	0·312	65	0·347	7·4
38	9·5	10·2	9·1	1·2	6·5	5·4	0·390	63	0·325	9·7
45	8·7	9·6	9·1	0·9	6·3	7·0	0·298	48	0·331	7·6
54	8·6	8·9	8·7	1·1	5·5	5·0	0·380	47	0·345	9·5
43	8·6	9·0	8·7	0·9	6·5	7·2	0·308	38	0·342	5·8
39	8·8	9·4	8·8	1·0	5·5	5·5	0·386	34	0·386	6·1
37	8·8	9·5	8·8	0·5	3·0	6·0	0·154	29	0·308	9·7
35	8·8	9·5	8·8	0·5	2·4	4·8	0·208	27	0·416	11·3
42	7·6	8·3	8·2	0·7	4·5	6·4	0·214	26	0·306	5·7
35	8·8	9·6	8·9	0·7	4·5	6·4	0·360	25	0·337	5·6
37	8·9	9·8	9·0	0·7	3·2	4·6	0·236	23	0·337	7·1
35	8·7	9·0	8·6	0·6	3·8	6·3	0·248	21	0·413	5·5
34	9·0	9·7	8·6	0·5	1·9	3·8	0·184	19	0·368	10·1
35	8·7	9·3	8·7	0·5	2·1	4·2	0·204	19	0·408	9·1
36	8·3	8·7	8·6	0·5	2·6	5·2	0·150	18	0·300	6·8
33	8·7	9·4	8·6	0·4	1·8	4·5	0·146	18	0·365	9·7
42	8·1	8·8	8·2	0·3	1·3	4·3	0·108	17	0·360	12·7
43	8·5	9·0	8·3	0·5	2·6	5·2	0·174	16	0·348	6·0

TABLE I—*Contd.*

Saturation percentage	pH 1 : 5 Soil-water extract	pH 1:2·5 Paste	pH Saturation extract	E. Conductivity in millimhos/cm. 1 : 5 Soil-water extract	E. Conductivity in millimhos/cm. Saturation extract	(E.C. × 10³) S.E. / (E.C. × 10³) 1:5 Extract	T.S.S. %	Total soluble cations me/L	T.S.S. % / (E.C. × 10³) 1:5 Extract	T.S.S. % / (E.C. × 10³) S.E.
52	9·0	9·6	8·8	0·6	1·9	3·2	0·222	16	0·370	8·2
30	9·4	9·8	8·9	0·8	3·8	4·8	0·296	15	0·370	4·0
38	8·6	9·3	8·4	0·4	1·9	4·8	0·122	14	0·305	7·5
32	8·3	8·8	8·4	0·3	1·2	4·0	0·096	14	0·320	11·2
34	8·5	9·6	8·5	0·4	1·4	3·5	0·126	12	0·315	8·6
32	9·0	9·7	8·5	0·5	1·5	3·0	0·156	12	0·312	7·8
27	9·3	9·3	8·6	0·4	1·6	4·0	0·162	11	0·405	6·8
32	8·3	8·2	8·0	0·3	0·7	2·3	0·094	9	0·313	12·4
36	8·0	8·7	7·8	0·2	0·5	2·5	0·072	8	0·360	15·0
41	8·6	8·4	8·0	0·2	0·5	2·5	0·062	7	0·310	14·0
33	7·9	8·2	7·6	0·2	0·6	3·0	0·066	7	0·330	10·9
47	8·2	7·5	7·6	0·3	0·5	1·7	0·110	6	0·367	12·0

With a view to present more precisely the inter-relationship between different estimations including the electrical conductivity of saturation extract, 1:5 soil-water extract as well as total soluble salts for different salinity categories of soils, the values as worked out for different salinity groups have been presented in Table II. The

TABLE II. RELATIONSHIP BETWEEN ELECTRICAL CONDUCTIVITY AND TOTAL DISSOLVED SOLIDS OF SOILS

Soil-salinity groups	No. of samples	T.S.S. %	Total soluble cations me/L	(E.C. × 10³) S.E. / (E.C. × 10³) 1 : 5 Extract	T.S.S. % / (E.C. × 10³) 1 : 5 Extract	T.S.C. / (E.C. × 10³) S.E.
1. High-salinity group						
SO_4-Cl (75—300 mmhos/cm.)	17	6·6	2386	11·76±0·900	0·358±·013	10·77±0·913
CO_3-HCO_3 (12—65 mmhos/cm.)	10	0·9	299	11·20±2·529	0·335±·020	9·76±1·639
2. Low-salinity group						
CO_3-HCO_3 (<12 mmhos/cm.)	33	0·2	27	4·95±1·812	0·347±·033	8·72±2·601
Average for all samples	60	2·0	74	7·94±3·752	0·348±·043	9·47±2·282

deviations from values of mean have also been calculated for each deduced relationship and presented along with the average values of the ratios.

The values in respect of relation between total soluble salts and the electrical conductivity of 1:5 extract are remarkably constant. The average ratio of 0·348 between these two determinations for saline-alkali soils of Gangetic alluvium differed only slightly from Piper's ratio of 0·375 and Hoon, Malhotra and Jain's ratio of 0·314. The drift from Piper's value further narrows down in light-textured soils of higher salinity range, predominating in SO_4-Cl ions, where a ratio of 0·358 has been found. In soils of the same salinity category, but predominating in carbonate-bicarbonate ions, the derived ratio is more diversified and the conversion factor is considerably lower than the factor obtained from overall average. Since the deviations from mean are not very broad, it can be safely suggested that the values of percentage of total soluble salts can be utilized to compute the values of specific conductance of 1:5 soil-water extracts and *vice-versa* in different types of saline-alkali soils of the Indian Gangetic alluvium by using the conversion factor given in Table II.

Once the value of electrical conductivity for 1:5 soil-water extract has been obtained, this value could be converted to electrical conductivity for saturation extract by using the factor given in Table II. As an independent check on this computed value, the electrical conductivity of saturation extract should also be calculated from the values of total soluble cations (which is equivalent to total soluble anions) to see if a reasonably near approach has been obtained.

The average relationship between total cationic concentration and the electrical conductivity of saturation extract has been worked out as 9·47 for the entire lot of samples examined in the present investigation. This value, though remarkably different from values obtained in an earlier publication by Agarwal and Yadav (*loc. cit.*), shows a closer resemblance to the values obtained by the workers of the U.S. Salinity Laboratory. This discrepancy may be possibly due to the inclusion of a large number of characteristically different saline-alkali soils covering all possible ranges in salinity ratings. However, as soon as the relationship is separated for different salinity soil classes, marked differences are observed between the values of the American workers and those obtained in the present investigations. Highly saline soils yield higher ratio than the lower salinity soils, the values being 10·77 in the former class, showing the nearest approach to the values reported in earlier investigations (Agarwal and Yadav, *loc. cit.*) and 8·72 in the latter class of saline-alkali soils. High-salinity class of soils, predominating in carbonate-bicarbonates, yield values of about 9·76, being the nearest approach to the values recorded by American workers.

In view of the fact that in the laboratory techniques hitherto employed for characterizing and diagnosis of saline-alkali soils in India the values for total soluble salts and individual anions (CO_3, HCO_3, Cl and SO_4) are usually available, these could be employed with advantage to obtain the figures for electrical conductivity of saturation extracts to appraise salinity status for crop growth on the salinity and alkali-scale proposed by Agarwal and Yadav (*loc. cit.*). The computed value so obtained will be of great use for practical purposes of soil-testing and advisory work. No claims are made that the actual laboratory determination of electrical conductivity of saturation extracts in soils should be dispensed with. But, if, for some reasons, such values

cannot be obtained in the laboratory, the present relationship should be employed to compute the same for judging the salinity status on Agarwal and Yadav's scale (1956b). These relationships will hold best for loamy soils with a saturation percentage varying from 30 to 45. Application of the proposed relationships for some typical soils is given in Table III.

TABLE III. COMPUTATION OF E.C. (SATURATION EXTRACT) VALUES IN TYPICAL SOILS

Type of soil	pH (1:2·5) paste	Saturation percentage	T.S.S. % (1:5 extract)	T.S.C. (S.E.) from anionic-analysis	Calculated E.C. (1:5 extract)	E.C. (sat. extract) mmhos/cm. From E.C. (1:5) extract	From T.S.C.	Actual experimentally	Salinity alkali ratings on Agarwal and Yadav Scale
High-salinity, SO_4-Cl	8·9	32	9·9	3,412	27·7	310	315	300	Crops do not grow
High-salinity, SO_4-Cl	8·6	33	8·1	2,648	22·7	266	245	237	,,
High-salinity, CO_3-HCO_3	9·8	41	1·4	408	4·1	45	42·2	46	,,
High-salinity, CO_3-HCO_3	10·3	34	0·8	231	2·3	24·6	23·8	27·2	,,
Low-salinity, CO_3-HCO_3	8·3	32	0·1	14	0·28	1·5	1·6	1·2	,,
Low-salinity, CO_3-HCO_3	8·0	36	0·1	8	0·28	1·0	0·9	0·5	Crops grow Normally

The values given above exhibit a close similarity between the observed and the deduced values of electrical conductivity through the conversion factors obtained in the present investigations. These studies open up potential possibilities of converting the older data of soil—salinity studies and afford better interpretations in terms of modern concepts of diagnosis of the salinity status of saline-alkali soils.

Some of the conversion factors deduced from such studies have been compiled together and expressed below with a view to facilitate the inter-conversion of one factor into another for appraisal of soil salinity.

CONVERSION FACTORS FOR DIFFERENT SALINITY SOIL GROUPS

1. *High-salinity soil group*

   (a) *Sulphate-chloride-rich soils*

   (i) Specific conductance (S.E.): Specific conductance (1:5 extract)
   Specific conductance (S.E.)/11·76 = Specific conductance (1:5 extract)

   (ii) Specific conductance (1:5 extract): T.S.S. per cent
   Specific conductance $\times$ 0·358 = T.S.S. per cent
   or Specific conductance $\times$ 3,580 = T.S.S. in ppm.

   (iii) Specific conductance (S.E.): T.S.C.
   Specific conductance $\times$ 10·77 = T.S.C. in m.e./L.

(b) *Carbonate-bicarbonate-rich soils*

(i) Specific conductance (S.E.): Specific conductance (1:5 extract)

Specific conductance (S.E.)/11·20=Specific conductance (1:5 extract)

(ii) Specific conductance (1:5 extract): T.S.S. per cent

Specific conductance $\times$ 0·335=T.S.S. per cent

or Specific conductance $\times$ 3,350=T.S.S. in ppm.

(iii) Specific conductance (S.E.): T.S.C.

Specific conductance $\times$ 9·76=T.S.C. in m.e./L.

2. *Low-salinity soil group*

Carbonate-bicarbonate-rich soils

(i) Specific conductance (S.E.): Specific conductance (1:5 extract)

Specific conductance $\times$ 4·95=Specific conductance (1:5 extract)

(ii) Specific conductance (1:5 extract): T.S.S. per cent

Specific conductance $\times$ 0·347=T.S.S. per cent

or Specific conductance $\times$ 3,470=T.S.S. in ppm.

(iii) Specific conductance (S.E.): T.S.C.

Specific conductance $\times$ 8·72=T.S.C. m.e./L.

SUMMARY

Systematic investigations were carried out on a large variety of saline-alkali soils found in mid-eastern *doab* of Uttar Pradesh with a view to study interrelationship between values obtained by the old, orthodox methods of appraisal of salinity followed in this country, and the modern estimations now adopted more or less throughout the world. A number of very remarkable relationships have been worked out, and it has now become possible to convert various expressions of soil salinity measurements into one another on the basis of which the data collected by the older methods can be interpreted in terms of modern methods of diagnosis of soil salinity.

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REVIEWS

A Bibliography of Subterranean Clover, together with a Descriptive Introduction. Compiled by D. E. Symon, (Mimeographed Publication No. 1/196 of the Commonwealth Bureaux of Pastures and Field Crops, Hurley, Nr. Maidenhead, Berks., England) 1961. Pages 122. Price 12 sh. Paper Covers, cr. 4to.

As the chief sheep producing country in the world, Australia has always paid attention to pastures and pasture plants. The fact that the subterranean clover (*Trifolium subterraneum* L.) is cultivated on no less than 20 million acres of land in southern Australia, shows the importance of the plant as a fodder crop and as the most important agricultural plant in Australia. It has not only raised the production of fodder but has also contributed to the improved fertility of soil by increasing its nitrogen content. Thus, great tracts of poor soils have been improved by the growing of this clover. These facts have been well brought out in the introductory part of the *Bibliography of Subterranean Clover* compiled by the author of this little publication. After the introduction the book presents an exhaustive list of literature on the subterranean clover, divided into various parts. Parts I and V constitute the main body of the references published up to the middle of 1960. Part II contains a list of articles and letters, which appeared in newspapers and agricultural press between 1900 and 1924 and which gave publicity to the plant. Part III comprises a list of references prior to Linnean period and starts from as early as 1650. Part IV is a cross reference index with references grouped under selected subjects. This part enhances the value of the bibliography.

The thoroughness of the bibliography is reflected in the introductory description which spotlights the characteristic features of the plant, like its history and the botanical, ecological and agronomical characters. This descriptive introduction lifts the otherwise plain bibliography to a plane of interest and readable matter, giving an ordinary reader a fund of information about the plant and its importance in the agriculture of Australia. The booklet covers, besides the topics already mentioned, the physiology and nutrition, breeding and genetics, seed production, pests and diseases etc., of the plant. The author could have included a section in his introduction, dealing in detail, the uses of the fodder in its various forms. The bibliography will be very useful to the specialist on fodder plants. This booklet may be used as a type to prepare similar booklets on the fodder plants of this country.—S.D.

Cork and the Cork tree. G. B. Cooke. International Series of Monographs on Pure and Applied Biology, Botany Division, Vol. 4. Pergamon Press, Oxford, 1961. (Pp. i-xii and 1-121). 50 s.

Cork has a romantic place in the history of botany, as being the first plant material to be seen through the microscope by Robert Hooke in 1665. He introduced the term 'cell' and that was the beginning of the 'cell theory.' But the history

of cork itself, as a utility product, goes back much further. The oldest record of cork dates back to about 400 B.C., and the product was used many centuries earlier. And cork by its many uses, the chief of which is as bottle cork, is known to everyone and is taken for granted. But very few know where it comes from or how it is processed. The *Cork and the Cork Tree* provides the layman with all information about the product and the tree that produces it, and the research workers many new facts. However, one wishes that the author had also included some botanical description of cork as a tissue and its formation from cork cambium.

The author with his thirty years of experience in research on cork has given a wealth of information on the history, distribution, care and harvest of the cork tree, and the processing, manufacture and future of the cork which continues to be an important commercial commodity, notwithstanding, the competition posed by the entry of plastics and other synthetic materials in industry.

It is revealed that cork is a good insulation material and is extensively used in domestic refrigerators, deep-freeze cabinets, air-conditioning and cold storage warehouses. As an acoustic material, it has an important place in modern building construction. Among the miscellaneous cork products are listed cork tiles, shoe products and novelty items. Development and uses of composition cork, prepared from granulated scrap, and used pulp, in crown-caps as liners, shoe soles, printing industry and other articles are well described. Statistics on cork production and world trade is also dealt with. A new use suggested for the cork oak is its planting as an attractive evergreen shade tree in gardens.

The bulk of commercial cork forests are of natural origin and till recently its available supply was enough to meet the demand. But, since the last 60 years, the industrial uses of cork have increased so much that new cork areas have been developed by planting of cork oaks. Efforts to establish cork areas in the U.S.A. through the 'Mc Manus Cork Project' are described. A chapter on the diseases and pests of cork oaks might have been a good addition.

The book is well illustrated but some of the photographs are not reproduced satisfactorily. For want of contrast, many details are lost, e.g., in the picture on page 2, which draws particular attention to the stripping hatchet.

On the whole, the book is highly commendable in its scope and thoroughness. It brings within a single volume almost all we would like to know about cork, and will be found useful by the industrialist, cultivator and the research worker. This volume should be a valuable addition to any library.—S.D.

THE INDIAN JOURNAL

OF

AGRICULTURAL SCIENCE

(GENETICS AND BREEDING SUPPLEMENT)

CONTENTS

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ADVANCES IN PLANT GENETICS AND BREEDING IN INDIA

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WHEN one looks at the world around himself, he is apt to be impressed more with the diversity than with the unity of life. The millions of different species of animals and plants, ranging from viruses to man, are examples of the ability of protoplasm to adopt innumerable variations of structure without losing the basic structural key. Although the diversity of living material is an amazing fact, it is also true that all through this diversity there runs an astonishing unity of structure and function. This unity clearly reveals itself when one descends from the organismal to cellular level. All forms in which life is manifested follow the same laws of heredity propounded by Gregor Mendel over a hundred years ago and rediscovered in the early years of this century. The knowledge that the units of inheritance or genes are largely located on chromosomes has led to a detailed study of the submicroscopic structure and biochemical organisation of cell organelles. These studies have shown that deoxyribose nucleic acid (DNA) is the key molecule whose structure confers the hereditary uniqueness of the cell. This knowledge in turn has led to the planning of experiments of such elegance that cross-breeding is possible now at the molecular level and hybrid DNA molecules have been produced in micro-organisms. The recognition of the self-replicating gene as the elementary basis of life has also given a meaningful direction to research on the origin of life. Genetics has thus consummated the unification of biology, a process so bravely attempted by Darwin over a century ago.

Fundamental studies in genetics

While research carried out in Europe and North America has reached the stage when there is a possibility that each phenotype can be denoted as an exact sequence of aminoacids in protein and each genotype as a corresponding sequence of nucleotides in DNA, little work was done in India until recently on the structure and biochemical organisation of the gene. Two factors appear to have been responsible for this situation. First, genetical research and teaching have been largely confined to agricultural colleges and research institutes where interest naturally centres round the application of genetical knowledge to the improvement of crop plants. Secondly, the pattern of university education prevalent in our country makes it obligatory for students to choose either biology or mathematics and not both from the very out-set of their career and consequently students of biology become inadequately equipped to work in the field of fundamental genetical research. These handicaps are now being resolved in institutions, such as the Post-graduate School of the Indian Agricultural Research Institute by the introduction of a greater flexibility in the choice of subjects and by providing the student with opportunities to take adequate supporting courses to compensate for the deficiencies in his early education. Besides agricultural institutions, biology departments of universities and medical colleges are recognising in a growing measure the importance and wider implications of genetics. A wing for fundamental genetical studies has also been set up at the Indian

Agricultural Research Institute. We can thus hope that in the near future genetics will develop to its full stature in India in coalescence with biometrics, biophysics and biochemistry.

Genetics and cytogenetics of crop plants

A large volume of valuable data has been collected by Indian workers on the genetics of rice, wheat, cotton, jute, tobacco, potato and many other crop plants. Several new linkage groups have been established and in the case of bread-wheat, the chromosomes carrying the genes conferring resistance to different rusts have been identified through monosomic analysis. Interesting work has also been done on the inheritance of quantitative characters in several economic plants. Indian workers have made important contributions in the fields of cytology and cytogenetics of crop plants and these studies have thrown light on the mode of origin of rice, bread-wheat, sugarcane, *Brassica* species, *bhindi*, mango and coconut. Thanks to the far-sighted publication policy of the Indian Council of Agricultural Research, much of the useful information relating to the work done in wheat, rice, jute, tobacco, cotton and coconut has now become available in consolidated monographs.

Progress in plant breeding

The advances made in India during the last twenty-five years in plant breeding have been summarised recently by Dr. B. P. Pal (*Empire J. exper. Agric.* **26:** 123-35, 1958). I shall, therefore, confine myself to giving an outline of some problems and welcome trends in plant breeding methodology.

Genetic variability

Naturally occurring variability: The success of a plant breeder's effort to evolve a superior strain of a crop plant depends on the spectrum of genetic variability available to him and on the use he makes of such germ plasm. Thanks to the co-operation of organisations, such as the F.A.O., and the Rockefeller Foundation, a large collection of genetic stocks is available in India in important crops like rice, wheat and maize. To carry out the introduction of plants in a systematic and scientific manner, the plant introduction scheme which has been in progress at the Indian Agricultural Research Institute since 1947, will be expanded and developed into a Bureau of Plant Introduction and Exploration during the Third Five Year Plan period. This will solve many of the difficulties faced by plant breeders in India in obtaining adequate genetic diversity in their breeding material.

Introductions from foreign countries are seldom useful directly, though there are some notable exceptions, such as the wheat variety, Ridley introduced from Australia. This is partly because climates and cultural conditions vary greatly from one region to another and a variety found suitable at one place may not perform equally well at another. Fortunately, we have in India a wide range of agroclimatic conditions and the superior genotypes introduced from outside the country can often be successfully utilized in some region or the other. An example of this situation is the adaptability shown by several exotic maize varieties, such as Amarillo de Cuba introduced from Mexico, and their consequent suitability for being used as parent strains to develop agronomically desirable inbred lines. Likewise, some Sea-island cotton strains (*Gossypium barbadense*) have been found to grow well in parts of Kerala, Mysore and Madras.

Attempts have also been made in recent years to explore and exploit the variability occurring within the country among varieties

and relatives of several crop plants. Thus, excellent collections of sugarcane (particularly *Saccharum spontaneum*) and rice (from the Jeypore tract of Orissa) have been made. Such a wide search has also led to the isolation of a strain of *bhindi* (*Abelmoschus esculentus*) possessing resistance to the yellow-vein mosaic virus, which is a major enemy of this crop. There is hence a growing awareness of the need to prevent an erosion of valuable indigenous germ plasm which inadvertently often accompanies progress in plant breeding. Ethno-botanical studies should be coupled with the evaluation of the primitive material for the desirable genes they possess, and we will then know where to look for certain genes. It should also be borne in mind that often a strain which by itself may not reveal the presence of desirable genes, may do so in hybrid combinations. The wild American species of *Gossypium*, for example, have practically bald seeds but when they are crossed with Upland cottons, it is seen that they contribute to the betterment of fibre properties. Hence, when introductions are made, evaluation of their potentialities will not be complete without a study of the characteristics of the hybrids between them and the local strains.

Induced variability: With the advent of the atomic era, sources of radiation have become readily available and hence many plant breeders in India have taken to mutation research. A gamma field radiation unit employing 200 curies of Co^{60} has been set up at the Indian Agricultural Research Institute and this facility is now open to all research workers in India. A smaller Co^{60} field unit is in operation at the Jute Research Institute, Barrackpore. In these "Gamma Gardens" the radiation response of a wide range of crop plants is being studied. We have a better idea now of the various parameters which control radiation sensitivity in plants and of the techniques by which mutation rate can be accelerated and lethality decreased. Methods are also known for ensuring that an induced mutant expresses itself phenotypically and does not get lost over a period of cell cycles. It is, however, true that a vast majority of the induced mutants are of negative economic value. This is because induced mutants most frequently arise from chromosomal changes, such as deletions. These changes have invariably an adverse effect in many plants, particularly diploids. Hence, very large populations will have to be screened and much regular breeding work will have to be undertaken to convert the raw mutant into a marketable variety. In most cases an induced mutant may be useful only for recombination breeding and the artificial induction of mutations should not hence be regarded as a substitute for any regular breeding process. This technique will be most useful when a single desirable trait is sought to be added to a strain, which is itself a product of good breeding. The fully awned wheat variety, NP 836, evolved at the Indian Agricultural Research Institute by irradiating the awnless strain, NP 799, is a good example of such a use. To obtain positive results in mutation breeding, a detailed knowledge of the genetic architecture of the plant, all phases of its ontogeny, the histogenetic aspects of the development of the inflorescence and the extent to which the plant can tolerate chromosome aberrations, will be essential. The work now in progress in India at several centres will provide within the next few years a better idea of the potentialities and limitations of this new research tool.

Breeding for resistance to diseases and pests

Much progress has been made in India in evolving varieties resistant to the important diseases of major crop plants. However, the

introduction of resistant varieties serves as a sieve for selecting strains of pathogens to which the new varieties succumb and consequently race changes continue to occur. The struggle between the breeder and the pathogen, thus becomes a continuous one with each trying to keep ahead of the other. To face the problem posed by the origin of new physiological strains in the fungi causing rusts, attempts are in progress at the Indian Agricultural Research Institute to develop a group of composite or multilineal varieties in wheat. These varieties will be a mechanical mixture of several phenotypically similar lines which will be genotypically different for rust resistance. Scientists attached to the Rockefeller Foundation's agricultural programme in Mexico have found that this approach may be a fruitful one.

Based upon the observation that new varieties bred for resistance to diseases invariably become susceptible later to new races and that among insects, strains resistant to insecticides develop more readily, the dictum 'breed plants resistant to insects and use fungicides against fungus diseases' has been advocated by some workers. This view has, however, not stood the test of time—new strains of the golden nematode, *Heterodera rostochiensis*, have arisen in Europe following the initiation of intensive breeding programmes to evolve potato strains resistant to this nematode. Hence, Indian breeders are adopting several approaches in tackling this difficult problem and are attempting to get genes from diverse sources of resistance combined in one genotype. Breeding for resistance to pests has been taken up only in recent years and research on the production of cotton strains resistant to jassids, and maize strains resistant to the corn-borer has made good progress.

Breeding varieties suited to intensive agriculture

The establishment of several river valley projects and new irrigation systems and the growing use of fertilizers by our farmers necessitate the development of varieties suited for intensive agricultural practices. A non-lodging habit and the ability to utilize the applied fertilizer for producing more grains will have to be essential qualities of the varieties of cereals evolved in the future. Already, several new Pusa wheats (NP 823, NP 824 and NP 828) possessing these attributes have been bred and distributed among farmers. Wheat varieties which yield well under rain-fed conditions have also been produced. In rice, attempts to achieve these objectives are being made through an extensive hybridization programme among the *indica* and *japonica* varieties. Several rice varieties capable of withstanding floods have been evolved. Attention is also being paid to breeding for better grain quality in wheat and other cereals.

Exploitation of hybrid vigour

Research carried out under the co-ordinated Maize Breeding Scheme has reached a stage when a suitable hybrid for each maize growing area will be available by 1962. Rapid methods of estimating combining ability are being devised and adopted. Good progress has also been made in evolving techniques for the economic production of hybrid seeds of jowar, cotton and vegetables, such as onion and tomato. Male sterile lines have been isolated in many plants and chemical methods of inducing pollen sterility have also been standardised for crops like cotton, onion and tomato. Thus, research both on the development of more efficient methods of breeding hybrid varieties in crops like maize and on the extension of the hybrid method to crops in which hybrid strains do

not yet exist, is in progress in India and we can expect that in the course of the next decade we may be able to tap fully the increase in yield possible from this source. A maximum increase in yield can be obtained by the interaction of hybrid strains with optimum conditions of growth, such as adequate moisture, high fertility and good drainage.

Breeding perennial trees

A significant development in the field of plant breeding in India in recent years is the formulation of breeding procedures based on sound genetic and statistical principles for perennial trees, such as coconut, arecanut and fruit and forest trees. These important economic plants offer, in view of their long life and heterozygous genotype, special problems as well as possibilities and the impact of the new procedures now being adopted will be felt after some years. Though cross-pollinated, considerable fixation of genes must have taken place in many coconut, arecanut and other populations, owing to crossing being confined to a relatively few individuals. Consequently, many populations suffer from what Dr. S. C. Harland has termed 'cryptic inbreeding depression'. Geographical race crossing for hybrid vigour is, hence, a must in such plants.

Statistical techniques in plant breeding

Statistical tools are to a plant breeder what a microscope is to a cytologist. The continuous improvements in statistical techniques are comparable to refinements in microscopy and result in an increased revelation of differences and confidence in interpretation. The growing use of precise statistical methods in plant breeding work in India is hence a good index of the advances taking place in this field. Indian scientists have evolved statistical methods of estimating heritable variability and its genetic component arising from the additive action of genes. Efficient breeding methods devised on statistical considerations, such as diallele crosses, polycross technique, mass pedigree system of selection, reciprocal recurrent selection, etc., are being used in the appropriate situations.

Co-operation of genotypes

It has been found recently by a research worker attached to the Indian Statistical Institute, Calcutta, that when two rice varieties are planted together, either fully mixed, in alternate rows or in separate halves of the same plot, each may influence the yield of the other. Similar findings had been made earlier by Swedish and Japanese scientists. This effect is sometimes favourable and it was observed in the rice experiments that co-operative interaction may increase the yield of a strain by as much as 26 per cent. The practical as well as the scientific significance of this phenomenon are obvious, though its theoretical basis is yet to be understood. The advantages and disadvantages of the mixed cropping systems, which have been in vogue in this country since ancient times, will have to be examined from this standpoint. The following statement of Prof. J. B. S. Haldane (*J. Genetics* **57:** 150, 1960) would merit the attention of all geneticists and breeders. 'One of the great differences between a tropical flora and a temperate one is the far greater number of species in the former (apart from very arid regions). Thus, India has over 20,000 species of flowering plants, a number far surpassing states of much larger area such as the U.S.S.R., and only surpassed by Brazil. One would expect symbiotic relations between flowering plants to be much commoner in regions with a diverse flora than in those where a natural plant community often consists of few members.'

Special techniques in breeding

Polyploidy breeding: Colchicine-induced autopolyploids have been produced in India in a wide variety of economic plants. Apart from ornamental and medicinal plants, this method has been of practical value only in fodder plants like *Trifolium alexandrinum* (berseem) and in the oilseed plant, *Brassica campestris* var. *toria*. Since naturally occurring polyploids are the products of a long period of evolution, the raw polyploids produced by the plant breeder will have to be given an artificial evolution before they are fit for commercial cultivation. Recognition of this fact led to the adoption of Harland's mass pedigree system of selection in *toria* and the polycross technique in berseem. In both these crops, there is a distinct possibility that tetraploids superior to the parent diploids in yield will become available for general cultivation in the near future.

Distant hybridization: Inter-specific hybridization has been commonly resorted to in India in the breeding of sugarcane, potato, tobacco, cotton and several other crops. Interesting inter-specific crosses have also been made in the genera, viz., *Oryza*, *Triticum*, *Sesamum*, *Brassica*, *Linum*, *Abelmoschus*, *Sorghum*, *Pennisetum*, *Manihot*, *Lycopersicum*, *Capsicum*, *Arachis*, *Coffea* and many others. An inter-generic cross involving the genera *Cajanus* and *Atylosia* has been made at Poona. Several such crosses have been made at the I.A.R.I., in Triticinae. Evolving varieties through inter-specific crosses takes more time but several promising hybrid combinations are available in India in the genera listed above. Recently, a cross between the two cultivated jute species, *Corchorus olitorius* and *C. capsularis* was successfully made at the Indian Agricultural Research Institute by using reciprocal grafting and pollen irradiation techniques. Attempts to make this cross were initiated in India as early as 1911, since each of these species possesses several desirable characteristics, particularly with regard to fibre quality, which will ideally supplement the needs of the other. However, systematic efforts to overcome the incompatibility barrier could be initiated only after it was found at the Indian Agricultural Research Institute that premature cessation of growth of the hybrid embryo is the cause for the failure of this cross. The F_2 progeny of *C. olitorius* × *C. capsularis* contained interesting recombinants and thus new possibilities have been opened up in jute breeding. This work also illustrates how a detailed knowledge of fertilization and embryo development helps in evolving techniques to overcome the incompatibility barrier in difficult crosses. Work on the *in vitro* culture of plant organs is also in progress at several centres in India and, hence possibilities exist for artificially culturing embryos prior to their abortion, which is a common cause for the absence of viable seeds in many inter-specific and inter-generic crosses. An interesting technique has been worked out at the Delhi University by which numerous replicas can be made of nucellar embryos in *Citrus*. By this means a promising genotype can be multiplied in large numbers.

Conclusion

The pace of progress in plant breeding research has become vastly accelerated in India in recent years owing to our ability to duplicate in a much shorter time several of the processes involved in evolution under the auspices of nature. As a result, improved varieties are fast becoming available and these can be profitably used to replace inferior ones in many crops. Agriculture can, however, reap the maximum benefit from the work of the plant breeder only if adequate arrangements are available for the multiplication and

distribution of seeds of improved strains. The steps already taken in the case of crops like potato and the Seed Corporation proposed to be set up by the Government of India for producing seeds of hybrid maize and other cereals would fulfil this need.

The association of genetics and breeding has led to the advancement of both. By studying a wide range of plants, the geneticist can choose the best material for his problem, while the breeder can turn to genetical knowledge for making the best of his material. This complementary relationship in needs as well as results renders it logical for fundamental studies to be carried out at the places where breeding work is already in progress. It augurs well for the future of these sciences in India that this principle as well as a spirit of co-operative endeavour among scientists belonging to different disciplines are taking practical shape in the various schemes sponsored by the Indian Council of Agricultural Research for the improvement of wheat, maize, sorghum, cotton, oilseeds and other crops.

COLOUR MUTANT OF RACE 15C OF BLACK RUST FOR ESTABLISHING GENE RELATIONSHIP WITH SOME OTHER RACES IN F_2

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THE establishment of relationships between the genes controlling the inheritance of different races of a particular rust is of considerable importance to a wheat breeder as he can plan his breeding work efficiently only after having a full knowledge of these relationships. To find out the relationships between the genes that govern resistance to two or more races carried by a common parent, it is customary to test the F_3 or F_4 lines with known reactions to a particular race. The reaction of these F_3 or F_4 lines is compared for these races and thus the relationship between them is easily established. This method, however, involves handling of huge populations, besides considerable encroachment on space in the glass-house. Further, the method also requires larger quantities of inoculum of different races for testing the material.

Difficulty also arises from the fact that the reactions of two races of the same rust cannot be distinguished from each other on the same plant. These obstacles can be overcome if the races under study could be distinguished from each other by their spore colour or by the nature of pustules produced by them on the plant body.

The 15C colour mutant, which arose as a mutation in biotype 15C of black rust (Misra and Lele, 1955), differs from other black rust races by the colour of its uredosori which are orange-yellow. Similarly, race 75 of black rust produces characteristic pustules with the epidermis intact and, hence, can be differentiated from other races of black rust.

So, advantage was taken of the 15C colour mutant in the present studies to find out whether it is possible to establish gene relationships between different races in the F_2 generation itself.

Material and methods

The F_2 generation of the intervarietal cross of *Triticum aestivum*, viz., Pb. C591 × NP 790 was selected for establishing the relationship between the genes carried by the NP 790 parent for resistance to *Puccinia graminis tritici* p.f. 75 and the 15C colour mutant.

The salient features of the wheat varieties and physiologic races of black rust used in this study are given below.

Pb. C591: A variety bred from the cross Type 8B × Type 9. Although a good yielder,

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it is highly susceptible to black and brown rusts and also to the loose smut disease.

NP 790: A variety evolved from the cross NP 165 × Thatcher by Pal at the Indian Agricultural Research Institute. It is highly resistant to all the races of black rust except race 122, and as such is a valuable source of resistance to wheat breeders in India.

Race 75: The collections made by Mehta in 1930-31 yielded race 75 apart from races 40 and 42. It is a weak race and not as virulent as the other races. It is characterized by the non-rupturing of the epidermis of its uredosori and can be distinguished readily from the other races.

The colour mutant of race 15C: A colour mutation in biotype 15C was observed in its 15th generation by Misra and Lele (1955). These workers did not observe any difference in the pathogenecity of the type race and its mutant when tested with 37 varieties of wheat and barley. However, they found differences in the germination percentage and incubation periods of the two. The uredosori of the mutant are dull orange in colour with the epidermis normally intact, while those of biotype 15C are dark-brown in colour with the epidermis rupturing soon after the appearance of the pustule. The uredospores of the mutant are smaller in size. The germination percentage and the growth rate of the germ-tubes of the mutant were found to be consistently less than the parental biotype. At higher (40°—87°F) and lower (39°—76°F) ranges of temperature, the mutant was found to have a longer incubation period than the parental race by one and three days, respectively (Misra and Lele, 1955).

To study the gene relationship between two races with reference to a particular cross, mixtures of the 15C colour mutant with the other races were tried. The 15C colour mutant was selected as one of the components of mixtures because of the ease with which it can be identified in a mixture.

The following different methods were tried for standardizing the technique of inoculation with mixture.

(i) Equal quantities of uredo-inoculum of the 15C colour mutant and a normal race were mixed and the seedlings inoculated. Although infection with both the races did occur, the method was found to be unsuitable because of the uncertainty that the inoculum of both the races got smeared on the leaf.

(ii) The first leaf of a seedling was inoculated with the colour mutant and the second with a normal race. Although the first leaf exhibited good infection, the infection on the second leaf was far from satisfactory. This method, besides being cumbersome, did not permit inoculation of the leaves with the two races at the same time.

(iii) Inoculating the upper half of the leaf with the colour mutant and the lower half with a normal race and *vice-versa.*

(iv) Inoculating the leaf first with the colour mutant and later with the normal race in order to avoid any irregular and unequal distribution of the inoculum of the two races.

Of the different techniques thus tried the last one was found to give satisfactory results.

The technique of inoculation and the methods followed to ensure infection, the recording of the reactions, etc., were the same as followed in normal glass-house studies.

The F_2 seedlings of the cross Pb. C591 × NP 790 were numbered and reactions for both the 15C colour mutant and race 75 recorded against each plant. Prior to this, the number of genes involved in controlling seedling resistance of NP 790 against these two races was separately established by inoculating the F_2 population with each of these two races individually. The data collected separately were checked up with the reactions of individual races in mixture.

Experimental results

The seedlings of Pb. C591 were highly susceptible to both the races (4 type), while those of NP 790 showed 0, and 1 type of reaction with the 15C colour mutant and immume reaction (0 type) with race 75.

One hundred and seventy-two seedlings were studied in the F_2 generation with the 15C colour mutant and it was found that Pb. C591 and NP 790 differ from each other by a single gene pair in governing reaction to 15C colour mutant (Table I).

TABLE I. REACTION TO THE 15C COLOUR MUTANT OF THE PARENTS AND F_2 GENERATION OF THE CROSS PB. C591 × NP 790

Parent or generation	No. of plants		Total	X^2	P. Value
	Resistant	Susceptible			
Pb. C591 ..	..	23	23		
NP 790 ..	27	..	27		
F_2 (observed)	42	130	172		
F_2 (expected) 1R : 3S ..	43	129	172	0·031	0·80–0·90

In the case of race 75, 188 F_2 seedlings were tested. It was found that NP 790 carried two duplicate dominant genes for governing resistance to this race. The data are presented in Table II.

TABLE II. REACTION OF THE PARENTS AND F_2 GENERATION OF THE CROSS PB. C591 × NP 790

Parent or generation	No. of plants		Total	X^2	P. Value
	Resistant	Susceptible			
Pb. C591 ..	..	19	19		
NP 790 ..	15	...	15		
F_2 (observed)	176	12	188		
F_2 (expected) 15R : 1S ..	176·25	11·75	188	0·0056	0·90–0·95

Having thus established the inheritance to these races separately, another set of 172 F_2 seedlings was inoculated first with the colour mutant and then with race 75. On the basis of the reaction to the two races at a time, the F_2 seedlings could be grouped into four independent classes as follows:

RM = Resistant to both (colour mutant and race 75).
Rm = Resistant to race 75 but susceptible to the colour mutant.
rM = Resistant to the colour mutant but susceptible to race 75.
rm = Susceptible to both (colour mutant and race 75).

The F_2 seedlings grouped into the above classes are shown in Table V. The expected frequency of these classes based on an independent assortment of the factors for resistance to the two races should be 15 RM: 45 Rm: 1 rM : 3 rm.

The data were analyzed (i) for confirming the inheritance to individual races, as was observed earlier in separate studies, and (ii) for studying the relationship between the two races, i.e., both the races at a time. The data of these observations are presented in Tables III, IV, and V.

TABLE III. REACTION OF THE F_2 GENERATION OF THE CROSS PB. C591 × NP 790 TO 15C COLOUR MUTANT IN MIXTURE

Generation	No. of seedlings		Total	X^2	P. Value
	Resistant	Susceptible			
F_2 observed	41	131	172		
F_2 expected on 1R : 3S ..	43	129	172	0·124	0·70–0·80

TABLE IV. REACTION OF THE F_2 GENERATION OF THE CROSS PB. C591 × NP 790 TO RACE 75 IN MIXTURE

Generation	No. of seedlings		Total	X^2	P. Value
	Resistant	Susceptible			
F_2 observed	157	15	172		
F_2 expected on 15R : 1S	161;25	10·75	172	1·792	0·10–0·20

The results of Tables III and IV are in conformity with those of Tables I and II, respectively, thus confirming the efficiency of the technique.

TABLE V. REACTION OF THE F_2 GENERATION OF THE CROSS PB. C591 × NP790 TO RACES 75 AND THE 15C COLOUR MUTANT IN MIXTURE

Generation	RM	Rm	rM	rm	Total
F_2 observed ..	38	119	3	12	172
F_2 expected on 15RM:45Rm: 1rM:3rm ..	40·3125	120·9375	2·6875	8·0625	172

X^2=2·122 P. Value=0·50-0·70

The above table shows that the observed data fit well into the expected ratio. Evidently, the genes for resistance to races 75 and the 15C colour mutant carried by NP 790 are completely independent of each other.

Discussion

The present studies were initiated to find out the possibility of establishing gene relationships between two races in the F_2 generation itself by using mixed inoculums of the races. Advantage was taken of the colour difference of a mutant of 15C of black rust. Of the different methods adopted for inoculating the seedlings, the one where the seedlings were first inoculated with the colour mutant and immediately afterwards with the race 75 of black rust was found to be the most efficient. Both the colour mutant and race 75 had practically the same incubation period and their reactions could be identified very easily.

The F_2 generation of an intervarietal cross of *T. aestivum*, viz., Pb. C591 × NP 790 was used for establishing the relationship between the genes governing resistance to these two races carried by the NP 790 parent. Three sets of F_2 seedlings of the above-mentioned cross were taken. One set was inoculated with race 75, another with the 15C colour mutant, and the third with both the races. Statistical analysis of the data with regard to the first two inoculations revealed that NP 790 carried two duplicate dominant genes for resistance to race 75, and a single recessive gene for resistance to the 15C colour mutant. On the basis of the reaction to both the races at a time, the F_2 seedlings were grouped into four classes, viz., seedlings resistant to both the races, susceptible to both the races, resistant to the colour mutant and susceptible to race 75, and resistant to race 75 and susceptible to the colour mutant. The analysis of the data so obtained showed that the genes for resistance to race 75 are different from those governing resistance to the colour mutant.

Though results of such studies are not met with in literature, Luig and Baker (1956) pointed out the possibility of usefulness of the colour mutants in such studies.

Apart from the 15C colour mutant, race 75 of black rust can be used in mixture with other races for establishing such type of gene relationships. Race 75 can be identified from other races by virtue of its nature of pustules. The pustules remain covered by a thin epidermal layer and the spores are not released even in highly susceptible reactions. Mutants of *Puccinia graminis tritici* p.f. 194 involving colour differences have been reported by Joshi and Kak (1955) and may be made use of in such studies.

Summary

The results of studies conducted to find out the gene relationship between two black rust races, namely, 75 and 15C colour mutant in the F_2 generation of the cross of resistant NP 790 and susceptible Pb. C591, are reported.

The resistance of NP 790 was dominant and controlled by two duplicate genes for race 75. NP 790 and Pb. C591 appeared to differ from each other by a pair of recessive

genes in conditioning reaction to the 15C colour mutant.

The F_2 generation of the cross when inoculated with a mixture of both these races gave the indication that the genes controlling resistance to these two races are independent of each other.

The efficacy of race mixtures in establishing gene relationships in the F_2 generation itself has been discussed.

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CYTOGENETICS OF PUNJAB WEEDS

I. CAUSES OF POLYMORPHICITY IN *CONVOLVULUS ARVENSIS*

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While considerable cytogenetical and genetical work on our major economic plants is being done in India, weeds, which are quite often troublesome and certainly unwanted in any cultivated field, have received no attention from these points of view. A study of such plants, besides being rewarding from a purely academic angle, is of practical importance since it will reveal the type of genetic system in operation, a knowledge of which would be helpful in any serious weed-eradication programme. It is with these points in mind that the senior author has been engaged in a study of weeds of the Punjab. Part of the results of this project, dealing with an investigation on the variation and evolution of an important weed (*Sisymbrium irio* Linn.) of the area, has already appeared (Khoshoo, 1960).

The present article is a short account of the studies on *Convolvulus arvensis* Linn. This species is a well-known polymorphic and persistent weed of cultivation, particularly in wheat fields of the Punjab. It is very difficult to eradicate, and is found not only throughout India (ascending up to 10,000 ft.), but also throughout the temperate and subtropical regions of the world.

The species has been under observation for some time and numerous selections from the Punjab plains, north-western Himalayas and also from Europe (in particular, Sweden, Denmark and Portugal) were cultivated at Amritsar while this department was temporarily housed in the Khalsa College. The cultivation under more or less uniform conditions removed the influence of the varied environments which the species ordinarily is subject to. As such, a direct comparison of the genotypes was rendered possible. Furthermore, progeny tests of several selections and reciprocal crossing between the selections from different habitats were made. While detailed account of this work will appear in due course, a short summary of our observations and conclusions on the interesting genetic system in the species is presented here.

Morphology

The cultivation experiments revealed that the species is polytypic. The various biotypes differ in almost all organs, viz., the stem, branches, leaves, peduncle and floral parts (in particular, the colour of the petals and stamens). The differences are both quantitative and qualitative. To illustrate the point, variability in the leaf may be as follows: the outline may be ovate, ovate-cordate, oblong to almost linear; the surface glabrous or nearly so; the apex obtuse and apiculate; the base auriculate or hastate or cordate or sagittate; the basal lobes acute to obtuse. Apart from such qualitative differences, there is extreme variability in length, breadth and thickness of the lamina and the length of the petiole (Fig. 1).

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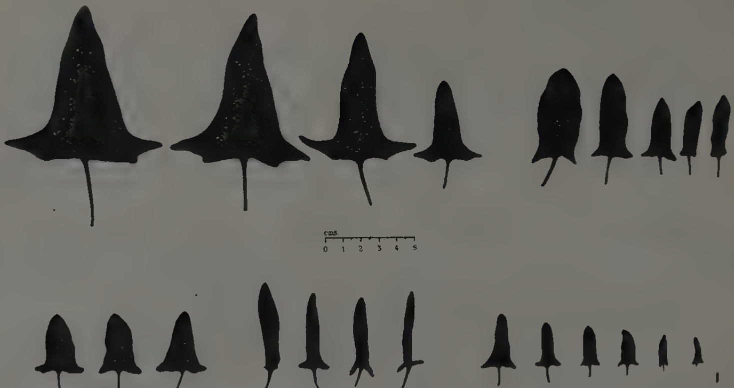

Fig. 1. Variability in leaf in the various selections (The diagrams have been drawn from comparable leaves).

Apart from the morphological differences exhibited by the various parts, the different selections also differ in physiological characters like the time taken for sprouting, flowering and maturing. In nature, on the morphological and physiological variations are superimposed the phenotypic modifications induced by environment. The more important of the environmental factors are the type of soil and its water content, light and temperature. This interaction between the genotype and environment results in an array of forms differing in morphology. It may, however, be pointed out that the nature and extent of modifications in this species are not as significant as in another common weed, *Sisymbrium irio* (Khoshoo, 1957, 1958). The manner of origin and preservation of variation in *Convolvulus arvensis* pose interesting questions. For this, the various selections were studied for their cytology and pollination and reproductive systems.

Cytology

The somatic and/or meiotic number was determined in most of the selections. The usual N HCl-aceto-orceine technique was used for determining the somatic chromosome number. For meiosis, flower buds were fixed in Carnoy's fluid in which the acetic acid component was saturated with iron acetate. Anthers were squashed in aceto-carmine.

All the selections worked out so far reveal 48 chromosomes in the root-tips and also in the tapetal cells (Fig 2). This number was corroborated by the presence of 24 bivalents during diakinesis (Fig. 3). At anaphase I, a normal segregation of 24 : 24 was observed. Up to three nucleoli were observed at late anaphase I or early telophase I; of these, two are usually smaller than the third. The subsequent course of meiosis is perfectly normal, resulting in normal pollen and seed fertilities.

Earlier, the species has been worked out by Wolcott in 1937 (cf. Darlington and Wylie, 1955), who reported $2n=50$. Possibly, he has made some error in counting because the authors have been unable to confirm this number even in the European selections available to them. Keeping the present results in view, it is possible that the species is based on $x=12$, which number has been suspected in *C. scammonia* by Heitz in 1926

(cf. Darlington and Wylie, 1955). Alternatively, the species may perhaps be based on x=8 (or even x=4), and the presence of three nucleoli sometimes seen during the late anaphase I or early telophase I is a pointer in this direction.

In conclusion, it may be pointed out that whatever be the basic number of *C. arvensis*, the number n=24 is polyploid in constitution. Furthermore, all the selections examined by the authors are homoploid at this level.

Pollination system

A study of the pollination mechanism shows that the flower is adapted for self-pollination which is usually the rule in the species. The species is perfectly self-compatible, and a good seed-set has been obtained from flowers that were self-pollinated. However, due to the fact that the flowers are quite big and rather showy, two types of butterflies have been observed to visit the flowers regularly during spring and early summer. Apart from the showy flowers, there is present a hypogynous disc which is nectariferous. The visit of the butterflies brings about a measure of cross-pollination, the exact extent of which is yet

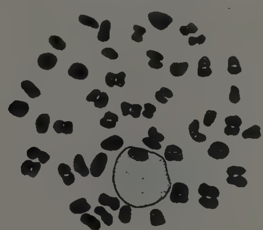
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Fig. 2. All the selections reveal 48 chromosomes in the root-tips and tapetal cells; this is confirmed by the presence of 24 bivalents during diakinesis (Fig. 3).
Fig. 4. A normal segregation of 24:24 at anaphase I × 1375.

to be determined. We have experimentally verified that there is no barrier to crossing between the various selections collected by us.

In brief, there occur both self- and cross-pollination, in particular the former. Evidently, this means that the progeny of an individual may not always be uniform. The hybrids, resulting after the cross-pollination by butterflies, would segregate in the subsequent generations even when self-pollination occurs in them. This has been experimentally verified by growing the progeny of individual selections after selfing or after open-pollination. The progenies obtained in this manner have been found to be morphologically rather heterogeneous.

Reproductive system

The seed obtained after self- or cross-pollination has been found to be 85-100 per cent viable, indicating that sexual reproduction is operative in the species. Besides this, small populations of seedlings have been found in nature by us. Along with the sexual reproduction, we have also found that the species reproduces to a very great extent by asexual reproduction by slender rhizomes. These rhizomes penetrate deep into the earth to a depth of one to three feet and are often almost beyond the reach of ordinary instruments of cultivation. Furthermore, the rhizomes are tenacious and can perpetuate even from a very small piece which soon sends forth trailing stems above ground. It is pertinent to stress here that the usual belief that the species reproduces only by vegetative means is erroneous, though, of the two types, it is more important.

The biological advantage of such efficient sexual and asexual reproduction is that it is helpful in preserving and perpetuating all variations arising out of recombination and sexual reproduction. Also, asexual reproduction vastly increases the dispersal potential of the species.

Conclusions

This species has all the important characteristics of a successful weed, since it has very efficient modes of dispersal, both sexual and vegetative. It can also stand a variety of climatic conditions, favourable and adverse. These properties owe their origin to an exceedingly successful genetic system, i.e., the inherent polyploid constitution of the species, the presence of self- and cross-pollination and the sexual and asexual modes of reproduction. Coupled with these is the total lack of barriers to crossability between morphologically dissimilar forms. Therefore, there arises polymorphicity due to recombination and segregation. Furthermore, the variants with an adaptive value arising from the above processes are preserved due to the efficient vegetative reproduction. The heterozygous variants form a constant source for the origin of new combinations. This phenomenon is repeated generation after generation and it is, therefore, not surprising to find *C. arvensis* an extremely successful weed even though it is homoploid in constitution.

In brief, the inbreeding-outbreeding system coupled with the vegetative-sexual reproduction and the polyploid constitution of the species are the important attributes that make it very well adapted to the variety of habitats exploited efficiently by the species. The inbreeding and vegetative reproduction ensure constancy and immediate fitness, while the outbreeding and sexual reproduction promote variability and ensure flexibility for adaptation to new situations. For any successful weed, there has to be, so to say, a compromise between the need for constancy for fitness as opposed to that for variability for flexibility. This has been achieved by *C. arvensis* in the manner detailed above even when it is uniform cytologically. However, in another successful weed of the Punjab plains, namely, *Sisymbrium irio*, similar

results have been achieved by a different genetic system: extreme plasticity or modifiability of the phenotype in response to the diverse environmental conditions in which it can grow almost obligate autogamy, very high seed production, auto- and allopolyploidy and efficient isolating mechanisms (cf. Khoshoo, 1961).

The several hundred individuals studied by us cytologically showed no meiotic irregularities. The interbiotypic hybrids raised by us also revealed no significant meiotic disturbances. It evidently reveals that major structural alterations in the chromosomes are not involved in the differentiation of the various biotypes. A logical conclusion emerges that differentiation within this weed is chiefly due to the gene mutations.

All taxonomists recognize this species as polymorphic, primarily because of the variability in the leaves. According to Fernald (1950), the "typical form has the sagittate- or hastate-ovate leaf, with acute basal lobes". He recognizes two forms: forma *cordifolius* Lasch. with a broad and cordate blade and rounded lobes, and forma *auriculatus* Desr. which has linear-oblong to lanceolate blades with acute auricles. However, for the present, we refrain to comment on the taxonomy of the species because of the obvious difficulties inherent with the type of genetic system present in the species.

Summary

Convolvulus arvensis Linn. is a well-known weed of cultivation in the Punjab and exhibits extreme polymorphicity. A chromosome survey of most of the selections of the species gathered from northern India and Europe persistently revealed the presence of 48 chromosomes in somatic tissue and 24 bivalents at diakinesis. This number has been inferred to be polyploid in constitution. The subsequent course of meiosis is perfectly normal, resulting in good pollen and seed fertilities. In view of the homoploid nature of the species, the causes of polymorphicity were looked for in the pollination and reproductive systems. It was found that in this species both self- and cross-pollinations occur. Coupled with this, both sexual and efficient vegetative reproductions are operative. There is a total lack of barriers to crossability between the various selections. Due to the recombination followed by segregation there arises polymorphicity. The variants are preserved by vegetative reproduction. The heterozygous variants form a constant source for the origin of new combinations. This process goes on generation after generation. The inbreeding and the vegetative reproduction ensure constancy and immediate fitness, while the outbreeding and sexual reproduction promote variability and ensure flexibility for adaptation to new situations. Such a genetic system makes the species a very successful weed not only all over north-western India, but also throughout the temperate and subtropical regions of the world. In the end, this system has been compared with the one found in another successful weed, *Sisymbrium irio*.

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EFFECTS OF SELECTION ON TETRAPLOID BLACK GRAM (*PHASEOLUS MUNGO* L.)

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THE effects of tetraploidy on five varieties of black gram have been studied by Sen and Chheda (1958). Considerable varietal difference in response to tetraploidy was observed, but in general the tetraploids grew slowly, had thicker, darker, green leaves, larger flowers and smaller pods with fewer seeds, though the seeds were larger and heavier. The C_2 generation plants were much more variable than the corresponding diploids specially with regard to the yield per plant. Several workers have shown that even in autogamous plants considerable improvement can be effected by careful selection, and in the allied plant green gram (*P. aureus* Roxb.) Sen and Murty (1960) could restore the decrease in several vegetative characters due to tetraploidy and partially increase fertility by selection alone. It is reasonable to assume that as most of our cultivated varieties are pure lines for a limited number of characters and as the genic balance brought in the diploid varieties through generations of selection is upset by the sudden induction of tetraploidy, selection pressure alone could restore the balance to a considerable extent. The tetraploids may be desirable for some other characters which make them better than the diploids, and the wider variability in tetraploids offers more scope for improvement through selection. Comparative studies on different characters with the diploid grown in randomized rows to find out the effects of two years' selection are given in this paper.

Material and methods

Ten high-yielding C_2 generation tetraploid plants of the T_9 variety were selected in their normal summer sowing time. As two generations of this variety can be grown in a year, half of the seeds were sown in the autumn season and the ten best plants selected from the C_3 population. Seeds of the diploid and selected tetraploids were sown in randomized rows, the tetraploids being of C_3 and C_4 generations. For all the morphological data, ten plants from each line were randomly sampled and all the measurements taken from these plants. The observations were on height, spread, flowering, pollen fertility and yield. The nitrogen content of the seed was estimated by the Kjeldahl method (A.O.A.C., 1955) and the crude protein content obtained by multiplying with the factor 6·25. For meiotic studies, buds were fixed in acetic alcohol (1 : 3) and smeared in aceto-carmine after mordanting in four per cent iron alum.

To find out the amount of variability present in different characters, their population variances were estimated and the significance of differences found out by the F test.

Observations

The tetraploid seeds germinated two to three days later than diploids. The percentage of germination in the tetraploids was also lower, about 76 per cent as compared to 99 per cent in the diploids.

Height: In the early stage, the plants of the C_3 generation were of significantly less height and those of the C_4 generation on par with the diploids. Though the C_3 generation plants surpassed the diploids on the 60th day, the C_4 generation plants were uniformly taller. On the 75th day, the difference in height between the diploids and the C_3 generation plants was not significant.

TABLE I. MEAN HEIGHT OF PLANTS AT SUCCESSIVE STAGES (IN CM.)

Ploidy	Days after sowing			
	30	40	60	75
Diploid	$10 \cdot 70 \pm 0 \cdot 30$	$26 \cdot 01 \pm 1 \cdot 22$	$31 \cdot 17 \pm 1 \cdot 19$	$33 \cdot 10 \pm 1 \cdot 27$
Tetraploid—C_3	$8 \cdot 18 \pm 0 \cdot 30$	$23 \cdot 80 \pm 1 \cdot 05$	$36 \cdot 21 \pm 1 \cdot 70$	$36 \cdot 89 \pm 1 \cdot 78$
Tetraploid—C_4	$10 \cdot 62 \pm 0 \cdot 32$	$29 \cdot 02 \pm 1 \cdot 01$	$43 \cdot 65 \pm 1 \cdot 48$	$44 \cdot 68 \pm 1 \cdot 82$

Only on the 60th day the variances of both the tetraploid generations were significantly greater than those of the diploid.

Spread and number of branches: The spread of the plants measured on the 75th day showed a significant reduction in the C_3 generation, the C_4 generation plants being on par with the diploids. Individually, some of the tetraploid plants of the C_4 generation exceeded the best of the diploid ones.

The mean number of branches on the main stem was significantly more in the diploids than in the tetraploids. The slight increase in the number of branches observed in the C_4 generation over the C_3 generation plants was not significant.

The variances of the tetraploids of both the generations were greater than those of the diploids, but no significant difference was observed in those variances for the total number of branches.

Flowering: The mean flowering time, in days, after sowing was significantly earlier in the diploids and the flowering range narrower. There was a wide variation in the date of the first flowering among the tetraploid plants. The flowering period was also much more prolonged in the tetraploids. The variance of flowering time was significantly greater in the tetraploids than diploids.

The percentage of stainable pollen in the diploid plants was about 99 compared to 80 per cent in the C_3 generation and 84·2 per cent in the C_4 generation, the difference between the C_3 and C_4 generations being not significant.

Fruits: Fruit production in relation to the total number of flowers was estimated by taking out three inflorescences at random. It was seen that whereas more flowers per inflorescence bloomed in the tetraploids, a less number of fruits was set.

Fig. 1. Tetraploid carkablmg

The total number of fruits and seeds per plant was much lower in the tetraploids. The fruit size was also smaller and there were less seeds per fruit. When fruits with an equal number of seeds were compared, those of the tetraploids were larger in size. The C_4 generation plants gave more fruits than the C_3 plants but the difference was not significant.

The seed yield per plant was considerably variable both in the diploids and tetraploids. The difference in variability was significant only between the diploids and the C_3 tetraploids. However, the estimate of joint variances of the C_3 and C_4 tetraploids differed significantly from that of the diploids. The coefficient of variation estimated was the highest in the C_3 generation tetraploids followed by the C_4 generation and then diploid. The differences in the variances between lines within the same treatment were non-significantly different for each of the treatments—diploid, C_3 and C_4 for yield data. Thus the significant differences between the variances on any two of the treatments were due to some inherent cause beyond soil heterogeneity.

Table II. Average spread, number of branches on the main stem, time of first flowering, flower and fruit numbers per inflorescence and fruit and seeds per plant

Characters	Diploid	C_3 tetraploid	C_4 tetraploid
Spread in cm.	45·70±1·001	39·65±1·312	46·64±1·350
Branches on main stem ..	3·85±0·121	3·02±0·122	3·26±0·120
First flower in days	35·70±0·263	47·60±0·397	45·10±0·317
Flowers per inflorescence ..	7·60±0·251	12·51±0·298	10·77±0·280
Fruits per inflorescence ..	3·61±0·172	1·60±0·101	1·95±0·110
Fruits per plant	84·40±2·721	24·90±3·840	33·04±3·322
Fruit length in cm.	3·78±0·052	3·38±0·008	3·44±0·081
Seeds per fruit	5·80±0·280	3·10±0·292	3·40±0·290
Yield per plant in gm. ..	13·00±0·320	2·70±0·361	3·70±0·411

Table III. Variability in grain yield

Source	D.F.	Variance	Coefficient of variation
Diploid	39	4·30	15·8
C_3 Tetraploid	49	8·55	96·9
C_4 Tetraploid	49	6·50	78·1

As regards fruit production, three types of plants were observed within the tetraploid lines: (i) completely fruitless, (ii) with a few fruits, and (iii) with a good number of fruits. The percentages of such plants were 2·7, 24·0, 73·3 in C_3 and 9·0, 10·0, 81·0 in C_4 lines. It was also observed that within the tetraploid rows there were several plants which were conspicuous for their high fruit production. Even in them the seed yield was little more than half those of the diploid plants, though the tetraploid seeds were heavier.

Fig. 2. Fruitless plant in the tetraploid black gram line.

Protein content of seed: The average protein content of the diploid seed was $23 \cdot 5 \pm 0 \cdot 10$ per cent and that of the tetraploid $24 \cdot 8 \pm 0 \cdot 09$ per cent, the difference between them was significant.

Meiotic study: Meiotic analysis of the cause of the difference between the tetraploids which set a good number of fruits and the ones which set very few or no fruit did not reveal any conspicuous difference. In both the groups the frequency of bivalents was quite high and comparatively few quadrivalents, trivalents and univalents were seen. The low-yielding plants tended to have more univalents and, though it could not be confirmed, some of them appeared to be aneuploids.

Discussion

Selection for two generations in the colchicine-induced tetraploid of an improved variety of black gram T_9 has resulted in a progressive improvement of several of the characters which suffered due to the induction of tetraploidy, though at a very slow pace. Due to the high variability of seed germination among the plant to row progenies, no effect of selection could be seen on the percentage of germination. Hagberg and Ellerstrom (1959) showed that in tetraploid Steel rye, screening of defective seeds decreased the high frequency of the aneuploid plants, resulting in better germination and increased yield. In black gram tetraploid progenies, the few low-yielding plants studied were found to have 44 chromosomes. The aneuploids may be inviable, thus reducing the germination percentage, though it is possible that we might have failed to detect the ones which grew, and a detailed study of a larger population might reveal them. Anyway, the effect of screening less-developed seeds on the germination percentage should be studied to know whether these seeds are the source of aneuploids.

A slower rate of germination, growth and development appears to be the characteristic feature of the autotetraploids and is seen here in late germination, reduced initial height and fewer branches, late flowering, etc. A slower rate of metabolism is often believed

to be the cause, but the real answer still seems to be a long way off. Whether this can be rectified by proper breeding or we shall have to be satisfied with increased yield through the longer growing period exhibited by the autotetraploids, remains to be seen.

That the meiotic irregularities are not pronounced enough to explain the reduced seed-setting in the tetraploid black gram, was also observed by Sen and Chheda (1958). In this study, a meiotic analysis for the cytological basis of difference between the tetraploid plants which give a good number of fruits and the ones which are practically fruitless, did not reveal anything either in their pairing behaviour or in abnormalities due to the chromosomal distribution.

From the high percentage of stainable pollen in the tetraploid black gram it seems that gametic abortion plays an insignificant part in the reduced fruit- and seed-setting. Between the other major factors for reduced fertility, Sen and Kawalkar (1960) showed that post-fertilization abnormalities, which are mostly pronounced in endospermous seeds like rye (Hakkanson, 1953), are not of much significance in black gram tetraploids, and that lack of fertilization due to a slow growth of the pollen tube was the major cause. One would wonder, whether selection pressure alone would suffice to restore the reduced fertility. The results show that the C_4 generation plants gave 37 per cent more seeds per plant than the C_3 plants, although the difference was not statistically significant due to high variability. It is possible that through generations of selection fruitless plants will be eliminated and also the frequency of the low-yielding plants will be reduced to get more seeds out of the tetraploid populations and the variance will be reduced, but that may not be enough to surpass the diploid level.

Without resorting to any other breeding method, it may be possible to increase the fruit-setting by finding a suitable type of cultural environment to restore the reduced fertility or by the application of chemicals that increase pollen germination and hasten the pollen-tube growth, among which boron is important (Batjer and Thompson, 1949; Gauch and Duggar, 1954; Pisarev and Zilkina, 1956). If the tetraploids respond to boron fertilization, spraying of the tetraploid field will not be a difficult or expensive proposition, considering the advantage of bolder protein-rich seeds of the tetraploid.

Summary

Comparative studies among diploids and progenies of high-yielding tetraploid plants of the C_2 and C_3 generations grown in randomized rows showed that, in general, the tetraploids had delayed and poorer germination, better height, less spread and fewer branches. The flowering was delayed and prolonged, and more flowers per inflorescence were produced. The fruit-setting was low, the fruits were smaller with larger but bolder seeds. The variability in all these characters was the highest in the C_3 generation and then in the C_4 generation tetraploid, and the minimum in the diploids. The tetraploid plants selected for high yield gave a highly variable progeny—from plants without any fruit to plants with a fairly good number. Meiotic studies failed to establish any correlation between the fruit-setting and the frequency of quadrivalent formation or any chromosomal irregularities among the low-fertility and the high-fertility plants. The average fruit-setting and seed yield increased due to selection, though the difference is statistically non-significant due to the high coefficient of variability. The crude protein content of the tetraploid seeds was significantly higher than that of the diploid.

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INHERITANCE AND LINKAGE IN *CAJANUS CAJAN*

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The inheritance of normal leaf in *Cajanus cajan* (L.) Millsp. has been studied and reported by the authors in an earlier publication (1960). So far, no report on the inheritance of cotyledonary leaf in this crop is available. Our studies on the inheritance of cotyledonary leaf and linkage relationships are presented here.

Material and methods

Technique of hybridization: The technique of hybridization adopted in securing hybrid seeds is slightly different from that reported by Mahta and Dave (1931). According to them, emasculation of buds is done in the evening at four and pollination is carried out the next morning. In this method, it is observed that majority of the emasculated buds either fall off next morning or they are unsuitable for pollination. In our technique, emasculation of the buds was done in the morning between seven and nine, and immediately after about half an hour hand-pollination was carried out. The seed-setting was found to be 35 per cent. In our method, care has to be taken in selecting proper-sized buds with receptive stigmas. It should also be seen with a lens that no pollen is sticking to the stigmatic surface, and if there be any, it should be washed off with a jet of water. Some of the hybrid seeds were also secured by the method of Mahta and Dave, and the percentage of setting obtained with it was 23.

Crosses studied: The crosses, which were used in the study of the inheritance of the normal leaf (Deshmukh and Rekhi, 1960) have been studied for inheritance of cotyledonary leaf. A description of the characters of the varieties used in the crosses in the present study is given in Table I.

TABLE I. DESCRIPTION OF THE NORMAL LEAF AND COTYLEDONARY LEAF

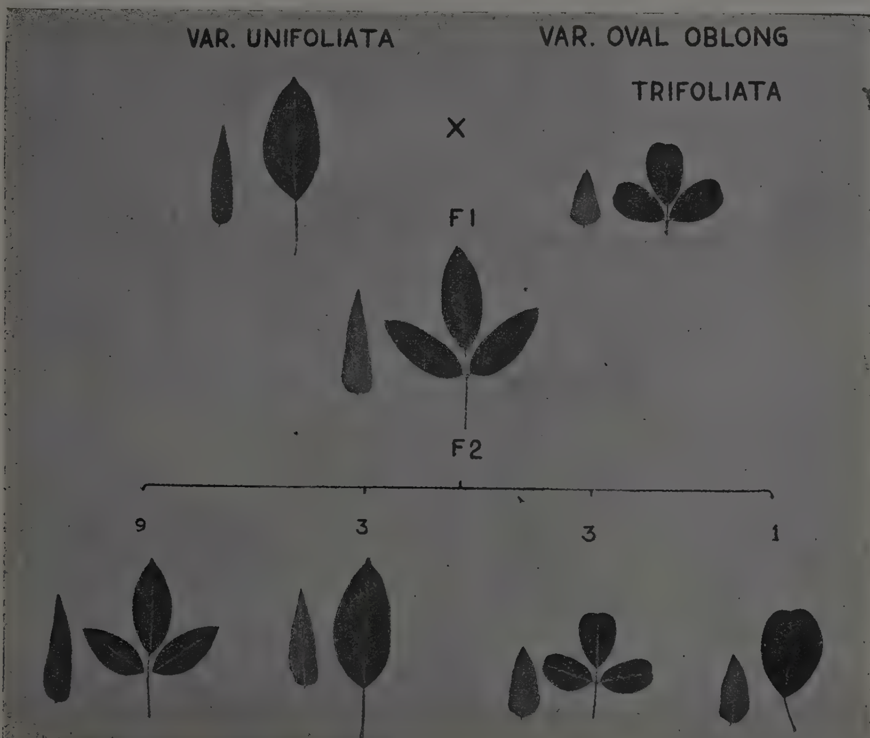
Variety	Leaf			Remarks
	Normal		Shape of cotyledonary leaf	
	Condition of leaf	Shape of apex		
No. 56	Trifoliate	Pointed	Lanceolate	
Hyderabad	,,	,,	,,	Reported by Joglekar and Deshmukh (1958)
Var. *unifoliata*	Unifoliate	,,	,,	
Var. *ovaloblong trifoliata* ..	Trifoliate	Roundish	Ovate type	

Observations

The crosses studied for inheritance of cotyledonary leaf, observations on the F_1 generation and segregation in the F_2 generation have been given in Table II.

TABLE II. OBSERVATIONS ON F_1 AND F_2 GENERATIONS OF THE CROSSES IN *C. CAJAN* (L.) MILLSP. FOR STUDY OF INHERITANCE OF COTYLEDONARY LEAF

Name of cross	Observations on F_1	Observations on F_2 behaviour: Observed: Lanceolate	Observed: Ovate Type	Calculated on 3:1 basis: Lanceolate	Calculated on 3:1 basis: Ovate type	Total X^2	P Between	Fit.
No. 56 × var. *ovaloblong trifoliata*	Cotyledonary leaf lanceolate	345	117	346·5	115·5	0·028	0·9 & 0·8	Excellent
Var. *ovaloblong trifoliata* × Hyderabad	,,	99	27	94·5	31·5	0·826	0·5 & 0·3	Good
Var. *unifoliata* × Var. *ovaloblong trifoliata*	,,	360	109	351·75	117·25	0·771	0·5 & 0·3	Good
Var. *ovaloblong trifoliata* × var. *unifoliata*	,,	686	236	691·5	230·5	0·174	0·7 & 0·5	Good



Plants possessing the pointed or acute apex of normal leaf showing the lanceolate cotyledonary leaf and those possessing the roundish leaf apex of the normal leaf showing the ovate type cotyledonary leaf.

It is observed from Table II that the lanceolate shape of the cotyledonary leaf is dominant over the ovate type, and that it is controlled by one pair of factors as evidenced from the monohybrid segregation on 3 : 1 basis in F_2.

Relationship between normal leaf and cotyledonary leaf from the F_1 and F_2 study: A study of interrelationship between the normal leaf and cotyledonary leaf and the parents of the F_1 and F_2 generations has indicated that the plants possessing the pointed or acute apex of normal leaf (unifoliate or trifoliate) possessed the lanceolate cotyledonary leaf and those possessing the roundish leaf apex of the normal leaf (unifoliate or trifoliate) possessed the ovate type cotyledonary leaf, as seen in the previous photograph. Not a single plant possessing the pointed apex of normal leaf with ovate type cotyledonary leaf or the roundish apex of normal leaf with lanceolate cotyledonary leaf in all the segregating population (1979 plants) of the four crosses mentioned in Table II, could be traced. This has suggested that the characters, (i) pointed or acute apex of normal leaf with the lanceolate cotyledonary leaf and (ii) roundish apex of normal leaf with ovate type cotyledonary leaf, are transmitted together indicating complete linkage.

It appears, therefore, that there is probably one pair of genes responsible for governing the two characters, viz., (i) pointed apex of normal leaf with lanceolate shape of cotyledonary leaf and (ii) roundish apex of normal leaf with ovate type shape of cotyledonary leaf. The other possibility could be that two independent pairs of genes, which are so closely situated on a chromosome that there is no possibility of any crossing over taking place in between them, may be responsible for the above two characters of normal as well as cotyledonary leaf.

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RECENT APPROACHES IN RICE BREEDING AND GENETICS

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THE production of rice can be raised by any or all of the following three means: (i) preventing loss through pests and diseases, (ii) providing suitable agronomic conditions to the growing crop, and (iii) using improved varieties for cultivation. While the adoption of the first two has its own limitations, the third still offers a great scope.

In the early years of rice research in India, evolution of high-yielding types through single plant selection from among the locally cultivated varieties in different states has formed the major preoccupation of the research staff. Therefore, a majority of the existing improved varieties of rice 416 out of 445, are the result of selection. Of late, the necessity of introducing new genes has been felt and hybridization projects have been initiated. While scattered attempts at hybridization between different varieties with the desired characters have been made in certain states, systematic hybridization projects have been initiated through the assistance of the F.A.O., and the Indian Council of Agricultural Research since 1950.

Suitable breeding material

The cultivated rice *Oryza sativa* has three recognized geographical races, viz., *indica*, *japonica* and *javanica*.

Javanica is confined to the Indonesian islands of the equatorial belt where the rainfall is well distributed throughout the year. The varieties are stout-culmed, tall, non-shattering with bold grains, mostly awned (and hence the name *bulu*, meaning bearded or awned) and are photo-insensitive under Indonesian conditions. As these varieties are accustomed to hot and humid weather, they are not able to establish successfully in either the temperate regions or in the monsoon lands.

Japonica is of recent origin and is said to have evolved from *indica*. It is largely grown in the temperate regions, such as Japan, Egypt, Spain and Italy. Varieties of this race are grown under heavily manured conditions with a liberal supply of irrigation water. They are adapted to low temperature and long photoperiods and give good response to manuring.

Almost all others, which do not come under the above two, are broadly grouped under *indica*. *Indica* is the earliest to have come under cultivation and, hence, manifests a wide range of variability in height, size and shape of grain, colour of husk and kernel, resistance to diseases and pests, etc. It is mostly confined to the monsoon-fed areas of the world. Varieties of this race are hardy and have wide adaptability to varying conditions, such as upland, hills, saline, deep water, floods, etc. Most of them are photo-sensitive and are perforce grown during certain periods of the year.

The *indicas*, when introduced into the temperate regions, come to head very late or do not attain the reproductive phase at all. The *japonicas*, when introduced into the monsoon lands, show a poor performance during the main crop season, being stunted in growth, forced to flower very early with extremely small panicles and showing a high degree of spikelet sterility. The *japonicas*

are, however, observed to be doing relatively better in the second crop season under Cuttack conditions, perhaps because of the low temperature prevalent during the major portion of its growth period.

Apart from the above three, there are various countries growing characteristic varieties of their own. For instance, in Taiwan, very high-yielding varieties, having only some characters of *japonica*, are grown. Similarly, there are very productive varieties under cultivation in the U.S.A., and these may be intermediate between *japonica* and *indica*. Although, attempts have been made to cross *japonica* with *indica*, the results obtained so far are far from satisfactory. They seem to be so widely divergent as to result in considerable amount of sterility when intercrossed. The inheritance of the desirable characters such as response to manuring seems to be intricate and selection for it has not been very successful.

It might, therefore, be worthwhile attempting hybridization between (i) Taiwan varieties and Indian varieties (*indica*), and (ii) *javanica* (Formosa) and *indica*. All these combinations are likely to offer a great range of variation in the segregating populations, and appropriate selections can be made to suit the Indian conditions. Hybridization between *indica* and *javanica* has been profitably utilized by Indonesia in evolving certain varieties superior to their existing *bulus*.

Direct introductions from Taiwan, the U.S.A., and some other rice-growing countries may also be worthwhile. Kaoshiung 22 and Taichung 65 have been successfully introduced into India from Taiwan and are reported to have given excellent performance at Almora. Ch. 10, Ch. 45, Ch. 1007 and Ch. 1009 are other instances of successful introductions.

The crossing of *indica* with *javanica* will have the added advantage of introducing photo-insensitivity into the *indica* varieties; this is felt to be a necessity in places where double or triple-cropping of rice during a single year is contemplated or is in vogue.

Utilization of genes from wild rices

In most rice-growing areas of the monsoon lands, the fields are swampy and illdrained. The poor conditions of drainage are not congenial for a healthy growth and bumper crop production. While ill-drained soils for rice cultivation may be unavoidable under certain circumstances, it may be worthwhile attempting to introduce genes from certain wild types which are adapted to waterlogged, ill-drained conditions and capable of smothering weeds (Sampath, 1960). Further, some of the wild types are resistant to insect pests and diseases and, hence, may offer scope for evolution of disease-resistant and insect-resistant types.

For instance, *Oryza ridleyi* from Malaya has been found to be resistant to the stem borer, one of the most common and destructive pests of rice.

Creating new rices: A wild rice from Cuba, originally classified as *O. cubensis* and subsequently reclassified as *O. perennis* by Chatterjee (1948), was crossed with the *O. perennis* from Orissa and the F_2 progeny studied by Madhavan Nair (1958). He found in the population a few plants having recessive characters of cultivated rice, viz., compact panicle, reduced awning and non-shedding grain. The subsequent F_3 and F_4 selections have given tall, erect plants approaching cultivated rice. This unexpected segregation from a cross between two wild rices can be interpreted in two ways. It may be an indication that *O. perennis* contains sufficient genetic variability to give rise again to cultivable types when crossed with another geographical race, or *O. perennis* var. *cubensis* is a *spontanea* of African origin, accidentally introduced into the West

Indies in the post-Colombian period. Whatever may be the interpretation, it seems to be a source of creating new rices which might be usefully exploited.

Technique of breeding

The chief aim of breeding has been the evolution of high-yielding types; the criterion of selection of plants from segregating generations of crosses has, therefore, been based on the yield. But the yield is a resultant effect of several factors, both genetic and environmental. Hence, this method of selection based on yield alone does not seem to be the correct approach.

Four methods of breeding rice are to be tested and the relative merits and demerits assessed.

Yield attributes like the number of ear-bearing tillers, length and density of the panicles, etc., will be kept in view while making selections. But greater importance will be attached to other factors like disease and insect resistance, photo-insensitivity, non-shattering habit, stiff straw, early flowering, etc., in the early segregating generations. The yield will be used as the criterion of selection only from the F_5 generation onwards.

There will be no selection adopted until the fifth generation. A sample of the bulked material will be grown in every generation. From F_5 onwards, desirable single plants will be selected and their progenies grown separately. The orthodox pedigree method will be followed thereafter.

There will be a selection of the best plants and the harvest of these will be mixed together, from which a sample will be taken for growing in the next generation. This process will be followed up to F_5. Thereafter, single-plant selections will be made and the orthodox pedigree method of breeding will be followed.

Single-plant selection will be done in the F_2 segregating generation and the orthodox pedigree method of breeding followed.

Convergent method of breeding: Richharia and Misro (1960) have mentioned the utility of a gene pool in the evolution of hybrid strains from *japonica* × *indica* crosses. One blast-resistant *japonica* (showing good response to manuring) was crossed with a flood-resistant type. Another drought-resistant *indica* was crossed with a salt-resistant type. The above two crosses A and B will be crossed between themselves and each of them with a hybrid between a non-lodging variety and an early, high-yielding Chinese type. Thus, a gene pool will be created and suitable selections having the desirable characters would be made, under different agroclimatic conditions.

Utilization of tetraploid rices

Tetraploid plants are produced by doubling the chromosome number of diploid cultivated plants, usually through colchicine treatment. The autotetraploids show a considerable amount of sterility, but the grain size is bigger and the protein content higher than that of the diploid. The tetraploids cannot stand competition with diploids and generally produce a few tillers. Therefore, if suitable agronomic conditions are evolved, the tetraploid rice crop may be able to give a yield commensurate with the cost of cultivation, and its yield may be useful in preparing various food products with a high nutritive value. If successful, the technique of tetraploid seed production and cultivation can be commercialized.

Perennating habit

The perennating habit in rice has been observed by various workers and the use of certain varieties as ratoon is prevalent in some states. It has been observed at this Institute (Richharia, 1960) that the pure, true-breeding, homozygous types showing

uniformity in flowering, grain size and some other economic features are segregating for the perennating habit. It is not yet known, whether any of the economic characters is associated with the perennating habit. It is contemplated to extract perennating and non-perennating types from the same pure strain and compare their yield and any other economic feature that may be associated with the perennating habit.

Utilization of haploidy in rice breeding

Recent work at the Central Rice Research Institute indicates that haploids occur very frequently in segregating populations of *japonica* × *indica*. Haploids can be isolated from the double seedlings obtained in the nursery beds of certain segregating cultures or even from pure types like GEB. 24 (Madras). Seed-setting in haploids can be induced by the hot water treatment at the time of fertilization. The haploids sometimes give rise to diploid sectors or may be treated with colchicine to double the number of chromosomes. In this manner, pure lines are being established.

Gamma-rays and radio-isotope treatments administered on the vegetative buds of the haploid plant may be able to induce structural changes, and these when doubled would result in new diploid plants.

Linkage

The cultivated rice (*Oryza sativa*) has the basic number of 12 chromosomes and, hence, is expected to have 12 linkage groups. The progress in establishing these linkage groups has been slow because of the following reasons: (a) The size of the chromosomes is small. The production of trisomics might have been found difficult and, hence, these are not used for linkage studies; (b) As each pollination gives rise to only one seed and the seed-setting in artificial crosses is as low as ten per cent, it is difficult to raise a sizeable back-cross population for linkage studies.

Out of about 300 genes studied in rice, only over 50 genes have been located in the different linkage groups. Recently, Nagao and Takahashi (1960) have established tentatively 12 linkage groups. But some of these have only one or two genes in each.

It is suggested that a diploid bearing a known dominant marker gene may be crossed with a tetraploid; the resulting triploid is expected to give plants with a chromosome number varying from normal diploid to triploid. Some of these might turn out to be trisomics, with the extra chromosome being the one bearing the dominant marker gene. From the phenotype, it should be possible to isolate the trisomic and use it for linkage studies.

There has not been any appreciable record of work with regard to the physiology of resistance of rice plants to saline, drought, flood and deep water conditions. Work on the physiology of saline-resistance and drought-resistance has been recently initiated at this Institute.

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KHARIF SONA A DAY-NEUTRAL SONAMUNG TYPE

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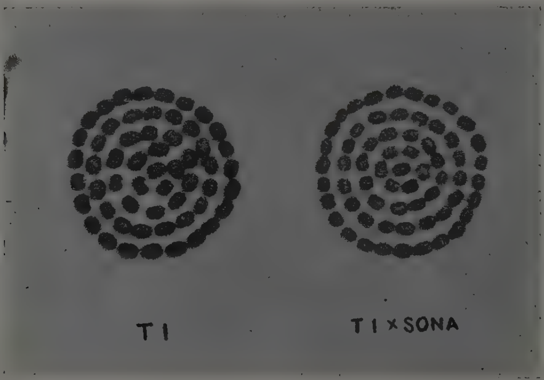
THE favourite Sonamung variety of green gram (*Phaseolus aureus*), with yellowish seed and aroma of the fried split seeds, can be grown only in the *rabi* season as it is a short-photoperiodic type. When sown in the *kharif* season (June-July), the plants continue to grow vegetatively for over 100 days and give a very poor yield. In the course of genetical studies in green gram undertaken in this laboratory, it was crossed with the day-neutral type T_1. Although the hybrid was intermediate in flowering and in several other characters, the green seed colour of T_1 was dominant, and from the segregating F_2 population three plants practically resembling the Sonamung but day-neutral, i.e., flowering in the *kharif* season, were selected. In the subsequent generations, the yellow seed colour and the desired aroma of the fried split seed of Sonamung plant and the day-neutral characteristics of T_1 parent could be fixed in a new variety. Besides, the plants were more uniform in flowering and maturity than T_1.

In order to find out the performance of the new variety–*Kharif Sona*–a yield trial was conducted during the *kharif* season of 1960 by using bulked F_4 seeds of selected plants of the $T_1 \times$ Sonamung progeny. The *Kharif Sona* plants were conspicuously different from the T_1 plants, being short statured and with more branches. The leaves were smaller and lighter-green in colour. Both the varieties flowered simultaneously in 32-35 days, and the harvesting could be completed in about two months. The pods were slightly smaller and conspicuously narrower, but there were more pods per plant. The seeds were smaller, lighter, and yellowish in colour. The plants resemble the Sonamung variety in all the characteristics. The *Kharif Sona* plants selected at random in the field gave lower yield per plant than the T_1 plants, and the yield per plot was also less.

The Sonamung plants grown alongside continued to grow vegetatively at the time when the T_1 and the *Kharif Sona* were

Two-month-old plants of T_1, *Kharif Sona* and Sonamung varieties grown in *kharif* season.

Variety	Pod (cm.) length	Pod (cm.) diam.	Pods per plant	Seeds per pod	Seed (mm.) length	Seed (mm.) diam.	1,000 seed weight (gm.)	Yield per plant (gm.)	Yield per acre (kg.)
T_1 ..	6·58	0·331	21·50	10·40	3·73	2·68	27·39	2·54	194·12
Kharif Sona	6·23	0·308	26·60	10·70	3·14	2·26	18·09	1·94	153·77



Seeds of T_1 and *Kharif Sona*

harvested. In the *kharif* season, it may be grown as a fodder legume but not for its seed yield.

Sonamung seeds command a premium for their colour and aroma of the fried seed, which often compensate for its lower yield as compared to many other improved *rabi* types. In *Kharif Sona*, even in the F_5 generation considerable variation still exists in the yield components, which offer opportunity for further improvement through selection. It ripens more uniformly than T_1 and the two pickings necessary for the T_1 can be avoided. In areas where Sonamung is popular, *Kharif Sona* can be grown as a catch crop in the *kharif* season.

INHERITANCE OF SEEDLING REACTION TO RACE 15C OF BLACK RUST AND ITS COLOUR MUTANT IN SOME INTER VARIETAL CROSSES OF *TRITICUM AESTIVUM* L.

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Of the 13 races and three biotypes of black rust prevalent in India, results of seedling reaction to eight races, namely, 15, 21, 24, 34, 40, 42, 75 and 117 and biotypes 21A and 42B have been reported earlier (Rao and Agrawal, 1960; Sikka *et al.*, 1961). Of the three biotypes, 15C is very virulent, and most of the varieties used as a source of black rust-resistance in India have been found to be susceptible to it. However, varieties like Kenya E 144, NP 789 and NP 790 are resistant. NP 789 and NP 790 are being used for breeding varieties resistant to this biotype.

The colour mutant of 15C was isolated by Misra and Lele (1955) from the 15th generation of 15C culture. The colour mutant differs from the original biotype in having dullorange-coloured uredospores as compared to dark-brown ones of the latter, and longer incubation period at both low as well as high temperatures. However, Misra and Lele (1955) did not find any difference in pathogenecity between biotype 15C and its colour mutant by testing 37 varieties of wheat and barley.

In this paper, results of the investigations carried out in respect of biotype 15C and the colour mutant of 15C are presented.

Material and methods

The material under study comprised the following intervarietal crosses of *Triticum aestivum*:

NP 790 × NP 710
NP 790 × NP 775
Pb.C 281 × NP 790
E 871 (Timstein × 2086. Sel. 1495A-1-31-2-1) × NP 718
E952 (Rio Negro) × Pb.C518
Pb.C 591 × NP 790

All the varieties used in these studies have been described by Sikka and Rao (1957) and Sikka *et al.*, (1961). In all these crosses, only the F_2 generation was studied. The first five crosses were studied against 15C, whereas only the last three crosses were studied against the 15C colour mutant.

The rust reactions of the varieties used in the present studies with biotype 15C and the 15C colour mutant are given below.

Variety	Type of reaction	
	15C	15C colour mutant
E871	3, 4	3, 4
E952	3, 4	0, ;
Pb.C 281	4	4
Pb.C 518	4	4
Pb.C 591	4	4
NP 710	4	4
NP 718	4	4
NP 775	4	4
NP 790	0, ; & 1	0, ; & 1

Note—E refers to the accession numbers for the exotic wheats at the Indian Agricultural Research Institute and NP refers to the improved strains bred at the same institute.

The authors' thanks are due to Dr. B. P. Pal, Director, Indian Agricultural Research Institute, New Delhi, and the Head of the Division of Mycology and Plant Pathology of the Institute for making available the inoculum of the races used in these studies.

The usual technique of inoculation and recording observations as described in an earlier paper (Sikka *et al.*, 1960) was followed in these studies as well.

Experimental results

Inheritance of seedling reaction to biotype 15C of black rust: The results of the mode of inheritance of seedling reaction to biotype 15C of black rust in five intervarietal crosses of *T. aestivum* are presented in Table I.

From the F_2 data given below, it would be seen that NP 790 carried a single recessive gene for controlling resistance to biotype 15C. In the crosses, E. 871 × NP 718 and E 952 × Pb. C 518, all the varieties were susceptible. In both the crosses, F_2 segregated into 3R : 13S, indicating thereby the operation of an inhibitory factor and a dominant factor for resistance.

Inheritance of seedling reaction to 15C colour mutant of black rust: The results of the mode of inheritance of seedling reaction in the three crosses to 15C colour mutant are presented in Table II.

TABLE I. INHERITANCE OF SEEDLING REACTION IN F_2 TO BIOTYPE 15C OF BLACK RUST IN FIVE INTERVARIETAL CROSSES OF *T. aestivum*

Cross	No. of plants			X^2	P. Value	Mode of segregation
	Resistant	Susceptible	Total			
Pb.C 281 × NP 790 ..	80	238	318	0·0043	0·90–0·95	1R : 3S
NP 790 × NP 710 ..	50	152	202	0·0066	0·90–0·95	1R : 3S
NP790 × NP775 ..	44	154	198	1·227	0·20–0·30	1R : 3S
E 871 × NP 718 ..	68	292	360	0·0045	0·90–0·95	3R : 13S
E 952 × Pb.C 518 ..	32	128	160	0·164	0·50–0·70	3R : 13S

TABLE II. INHERITANCE OF SEEDLING REACTION TO 15C COLOUR MUTANT OF BLACK RUST IN F_2 OF THREE INTERVARIETAL CROSSES OF *T. aestivum*

Cross	No. of plants		Total	X^2	P. Value	Mode of segregation
	Resistant	Susceptible				
Pb.C 591 × NP 790 ...	42	130	172	0·031	0·80–0·90	1R : 3S
E 871 × NP 718 ..	63	257	320	0·184	0·50–0·70	3R : 13S
E 952 × Pb.C 518 ..	97	39	136	0·98	0·30–0·50	3R : 1S

In the case of the cross Pb.C 591 × NP 790, a single recessive gene appears to govern the seedling reaction to the colour mutant. In the second cross, viz., E 871 × NP 718, both the parents were susceptible to this colour mutant. The F_2 segregation into 3R : 13S indicated the operation of an inhibitory factor and a factor for resistance. The resistance of E 952 (Rio Negro) appeared to be controlled by a single dominant factor.

Discussion

Five crosses were studied with race 15C of black rust. NP 790 in crosses with

Pb.C 281, NP 710 and NP 775 was found to carry a single recessive gene in controlling resistance. However, Rao *et al.*, (in press) reported that NP 790 carried two duplicate recessive genes, governing resistance to this race, in crosses with Pb.C 273 and Pb.C 518. In the crosses, E 871 × NP 718 and E 952 × Pb.C 518, a segregation ratio of 3R : 13S was obtained in the F_2 generation. These results may be explained on the assumption that E 871 and E 952 each carry an inhibitory gene along with a dominant gene for resistance to 15C, and that NP 718 and Pb.C 518 the corresponding recessive alleles.

Three crosses were studied with 15C colour mutant. NP 790 in cross with Pb.C 591 was found to carry a single recessive gene. NP 790 was found to carry (Rao *et al.*, in press) a single recessive gene in cross with Pb.C 281, whereas two duplicate recessive genes in crosses with Pb.C 273 and Pb.C 518, for governing resistance to the colour mutant. E 871 in cross with NP 718, carried an inhibitory gene along with a dominant gene, whereas E 952 in cross with Pb.C 518 a single dominant gene for governing resistance to the colour mutant.

On comparing the results obtained with 15C and its colour mutant, it would be seen that NP 790 and E 871 behave alike as regards their genetic constitution for governing resistance to both the races. However, E 952 with the cross Pb.C 518 gave an F_2 segregation of 3R : 13S with 15C and 3R : 1S with the colour mutant. This shows that E 952 (Rio Negro) carries a single dominant gene along with an inhibitor for controlling resistance to 15C, whereas only one dominant gene against the colour mutant. In other words, it may be said that the mutation has not only changed the colour of the spores of 15C but also its pathogenicity. This result is, therefore, at variance with that reported by Misra and Lele (1955). Probably the difference arises from the fact that these authors did not include E 952 (Rio Negro) in the material studied by them.

Summary

Results of the studies conducted to find out the mode of inheritance of seedling reaction to race 15C of black rust and its colour mutant in certain intervarietal crosses of *Triticum aestivum* are reported.

NP 790 was found to carry a single recessive gene for controlling resistance to both of them. Similarly, E 871 (Timstein × 2086 Sel. 1495A-1-31-2-1) carried a single dominant gene along with an inhibitor which impart resistance against 15C and the colour mutant.

However, the 15C colour mutant was found to differ in pathogenicity from 15C when tested on Rio Negro (E 952).

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INDUCED AUTOPLOIDS IN FERNS WITH SPECIAL REFERENCE TO COLCHI-AUTOTETRAPLOID IN *ADIANTUM CAPILLUS-VENERIS* L.

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CYTOLOGICAL investigations on ferns during the last one decade have revealed many species complexes and polyploid taxa (Manton, 1950; Mehra and Verma, 1960; Manton and Sledge, 1954; Wagner, 1955; Walker, 1955; Alt and Grant, 1960) which deserve critical analysis. The method of elucidation of the nature of polyploidy in *Nasturtium officinale* complex (cf. Howard and Manton, 1946) has now become a standard procedure. However, so far there has been no case in ferns where Howard and Manton's (*loc. cit.*) methods have been employed for analysis of genome relations and the nature of polyploidy. Perhaps, it may be due to the difficulties involved in raising autoploids in ferns.

Previously induced polyploidy in ferns involved an aposporous regeneration of the diploid gametophytes, usually from the first few excised leaves of young normal sporophytes (seedlings). Goebel (1908) was the first to report such regeneration in the members of Cyatheaceae and Polypodiaceae, and he got typical aposporously regenerated prothalli in *Osmunda regalis* L. Heilbronn (1910) was the first to obtain an autotetraploid sporophyte from an aposporously-regenerated prothallus in *Polypodium aureum* L. Similar investigations were followed by many enthusiasts, but in all such instances the regenerated prothalli did not proceed beyond the bearing of sex organs. Lawton (1932, 1936) outlined the entire method and produced four autotetraploid sporophytes experimentally, namely, in *Aspidium marginale* (L.) Sw., *Woodwardia virginica* (L.) Sm., *Cystopteris fragilis* (L.) Bernh. and *Osmunda regalis* (L.). None of these sporophytes reached maturity and, hence, meiosis was never studied.

Independently, Lang (1924) had induced apospory earlier in the young leaves of *Osmunda regalis* L., and the cytological investigations on both the aposporously regenerated prothalli and sporophytes produced by them were made by Manton (1932). Manton (1950) also synthesized autotriploids by using the normal haploid prothalli as the male parent. Manton (1950) alone has studied the cytology of the autotriploids and autotetraploids produced by aposporous gametophytes, and her main object was to point out the evolutionary significance of autoploidy and also to be able to interpret the cytological data of other polyploid ferns. It may be emphasized that this procedure (cf. Lang, 1924; Lawton, 1932, 1936; Manton, 1950) of obtaining aposporous diploid gametophytes bearing sex organs

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which would yield autotetraploid sporophytes, is rather cumbersome and is not always sure.

Induction of autoploidy by colchicine has not been tried successfully so far in ferns, although Mehra (1952), Yamasaki (1954) and Mehra and Loyal (1956) have studied the effect of colchicine on fern prothalli, while Mehra and Loyal (*loc. cit.*), have also laid stress on the abnormal spermatogenesis in polyploid prothalli. Panigrahi (1955), however, attempted the raising of an autotetraploid of the diploid *Cyclosorus repandulus* (v.A.v.R.) Ching by immersing young seedlings (sporophytes) at the three-leaved stage in an aqueous solution of colchicine, but could only get a sectorial chimera as evidenced only by the doubled chromosome number in the root-tips produced by a part of the treated plant.

Obviously, there has been little contribution regarding the induction of autoploids in ferns perhaps because of the apparent difficulties and limitations in following the traditional method of aposporous regeneration of diploid gametophytes. The authors are in the process of standardizing a technique for raising autoploids in ferns by colchicine treatment (cf. Verma and Loyal, 1960b), and, in the first instance, *Adiantum capillus-veneris* has been selected since it grows well in the Panjab plains, has a low gametic number (n=30, also diploid throughout its range: cf. Manton, 1950; Verma and Loyal, 1960a; Mehra and Verma, 1960) and possesses many garden varieties which probably indicate potentialities for genic mutations. Apart from the interest of the evolutionary significance of autopolyploidy in ferns, these studies would reveal the nature of pairing in autotriploids and autotetraploids and their fertility in reproduction. This would facilitate biosystematics of the various species complexes in Himalayan ferns. In addition, the raising of trisomics for genetical investigation and also of horticultural varieties is envisaged.

Methods

Before describing the procedure, it may be mentioned that in general, ferns possess a single apical cell in the shoot from the derivatives of which leaf primordia as well as roots are differentiated. Hence, once the apical cell gets doubled in its nuclear content, all the subsequently produced leaves (fronds) would be autopolyploids. Though apparently it seems difficult, but it is comparatively easier and quicker. In the present case, small actively growing rhizomes of *A. capillus-veneris* with their leaves intact were carefully uprooted and washed thoroughly under tap water. The rhizomes were not defoliated since the leaves would provide the requisite nourishment when the rhizomes were replanted after treatment. The rhizomes were treated in three different ways: (i) They were immersed in an aqueous solution of amorphous colchicine of 0·1, 0·2, 0·3, 0·5, 0·8 and 1 per cent for 24, 48 and 72 hours. After a careful washing following the treatment, these were planted in pots containing garden soil, and watered from below. Some rhizomes were treated similarly with distilled water, which acted as control; (ii) lanoline paste was applied to the tips of the rhizome with 0·2 and 0·5 per cent colchicine concentration, and the plants were never uprooted; (iii) five rhizomes were injected each with 0·2, 0·5 and 0·8 per cent aqueous colchicine near the growing tip with the help of a hypodermal syringe. Of the three methods described above, the authors have found the immersion in a 0·5 per cent solution for nearly 48 hours as the most successful.

Observations

A critical morphological and cytological comparison of the fronds produced by the

treated and untreated rhizomes has been made.

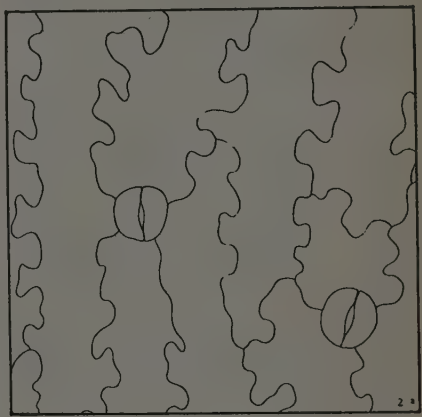
Morphological comparisons: The fronds produced by the treated rhizomes show a slow rate of growth as compared to the normal untreated ones, and the fronds in the former case also remain dwarf as compared to the diploids kept as control (Table I). The fronds are somewhat thicker and darker, but do not differ in shape. The ultimate pinnules have nearly the same outline (Fig. 1a, b). However, the number

Fig. 1. a. Fronds produced by treated rhizomes.
b. Fronds produced by untreated rhizomes.

of sori per pinnule and the number of sporangia per sorus show a decrease in the treated ones, but the spore size shows a well-marked increase over those of the untreated ones (Table I). A quantitative comparison of the fronds from the treated and untreated rhizomes was made, particularly for the epidermal cells and stomatal measurements. The cell size of the epidermis shows a slight increase in the autotetraploids (Fig. 2a and b). From the stomatal measurements of the treated and untreated fronds (Table II), it is apparent that the average dimensions of the normal diploid are $32 \cdot 6 \times 30 \cdot 6 \mu$ and those of the autotetraploid are $43 \cdot 2 \times 38 \cdot 1\ \mu$. This is a primary sign that the leaves on the treated rhizomes are polyploid and this could be safely employed for detecting other polyploid plants still in sterile condition.

Meiosis

Only a few fronds from the treated rhizomes became fertile. The sori at different stages of maturity were fixed in 1 : 3 acetic



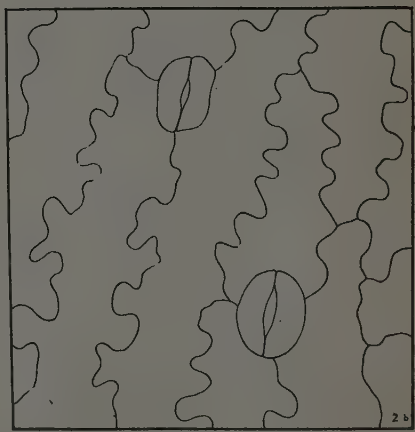


Fig. 2. Increased cell-size of epidermis in frond produced by treated rhizome (b) compared to an untreated one (a).

alcohol, and aceto-carmine squashes were made as usual. The untreated plants possess unequivocally a genetic set of 30 chromosomes at diakinesis (Fig. 3). In the case of the treated ones, relatively fewer spore mother cells have been analysed critically at diakinesis. Only quadrivalents and bivalents are observed (Fig. 4a, b), which as it appears can disjoin regularly with ease. An analysis of seven exceptionally clear spore mother cells at diakinesis reveals (Table III) that the number of quadrivalents ranges from 10-13 and bivalents from 30-40, the average associations being $12_{IV}+36_{II}$.

The quadrivalents are in the form of rings or chains and possess almost completely terminalized chiasmata at diakinesis. The high degree of terminalization may also augment the normal disjunction during anaphase (Fig. 5a, b). In only one case two bivalents showed early disjunction. Meiosis is perfectly regular. In all the cases examined, 64 spores result in a sporangium which are seemingly viable because of their well-filled nature (Fig. 6). It is of unusual interest to observe some of the spores (three to five per cent) in a sporangium with bilateral symmetry (Fig. 6) in contrast to the tetrahedral symmetry, usual for the species. This aspect of the present study is under investigation, but it seems likely that the aberration in symmetry is due to colchicine effect which in some way has disturbed the

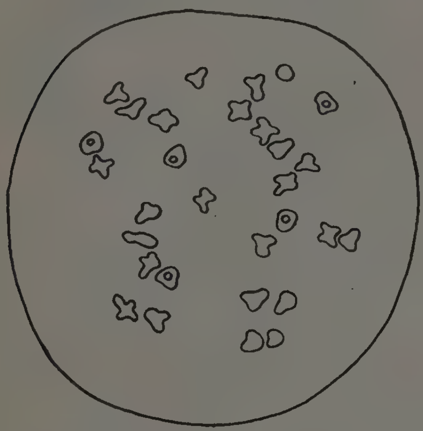


Fig. 3. Thirty chromosomes at diakinesis in an untreated plant

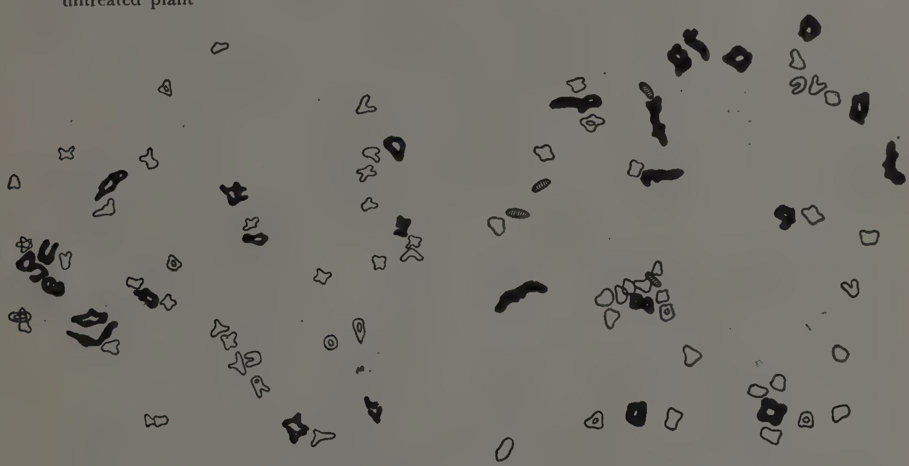


Fig. 4. Quadrivalents and bivalents observed in the case of a treated plant.

normal course of cytokinesis. The change in symmetry cannot be attributed to the polyploid condition *per se*, because no such aberration has been recorded in any of the polyploid ferns discovered so far in nature. It may be pertinent to mention here that Mehra and Loyal (1956) have observed another abnormality, namely, branched rhizoids, being caused by colchicine action in the sporelings of *Goniopteris prolifera* Roxb.

The resultant spores from an autotetraploid sporangium have been germinated and it has been found that nearly 80 per cent are healthy and viable. The study on gametophytes are in progress and would be discussed elsewhere.

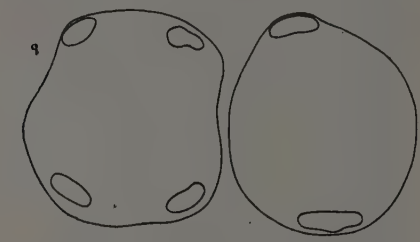


Fig. 5a. & b. The high degree of terminalization may also augment the normal disjunction during anaphase.

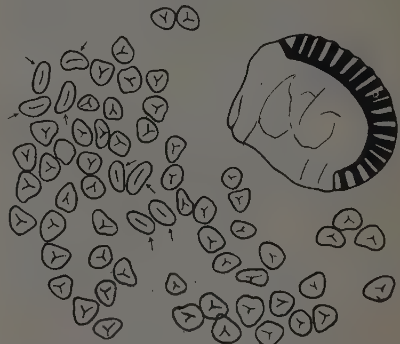


Fig. 6. Seeming viable spores because of their well-filled nature. Some of the spores possess bilateral symmetry.

Table I. Quantitative data

	Average length of fronds	Average thickness of pinnae	Average size of epidermal cells	Average spore size	Average number of sori per pinnule	Average number of sporangia per sorus
Treated ..	2–5″	129·5µ	216·0 × 50·2µ	41·3—49·2µ	1–3	3–7
Untreated ..	5–9″	124·2µ	195·6 × 50·0µ	38·5—41·3µ	4–7	8–17

Table II. Stomatal measurements

	Diploid			Autotetraploid		
	Length (µ)	Breadth (µ)	Number examined	Length (µ)	Breadth (µ)	Number examined
	30·6	30·6	25	40·8	37·4	25
	34·0	30·6	20	44·2	44·2	10
	32·2	30·0	10	51·0	40·8	15
	34·0	31·2	8	40·8	40·8	10
	30·6	29·0	9	45·9	32·3	13
	34·0	32·0	10	37·4	35·7	10
				45·9	35·7	20
Mean	c. 32·6	c. 30·6		c. 43·2	c. 38·1	

TABLE III. CHROMOSOME ASSOCIATIONS AT DIAKINESIS IN THE AUTOTETRAPLOID

Associations	Combinations observed				Total No.	Average No. per cell
Quadrivalents	13	12	11	10	84	12
Bivalents ..	34	36	38	40	252	36
No. of cells	3	2	1	1	7	

Discussion

The present findings reveal that the rhizomes can be treated successfully with colchicine in raising autopolyploids in ferns, and this method has some obvious advantages over the uncertain method of apospory. Herein, the meristem possesses a single apical cell which if rendered double in nuclear constitution will naturally produce subsequently fronds of polyploid nature. The resulting spores would normally produce diploid gametophytes which can be used for crossing with the natural tetraploids and diploids. Such a study would be helpful in biosystematics of various species complexes and analysing their genome relationships. Autoploid series would also be utilized for genetic studies in ferns especially by the use of trisomics. In addition, it would also help in raising certain horticultural types which would be propagated by vegetative means.

The autotetraploids, as expected, have shown gigas characters as evidenced by quantitative comparison. In the present case, the autotetraploid shows, on the average, 12 quadrivalents and 36 bivalents at diakinesis, i.e., 40 per cent quadrivalents and 60 per cent bivalents. These results are almost similar to the autotetraploid *Osmunda regalis* investigated by Manton (1950). In *O. regalis* too, mostly quadrivalents and bivalents are observed. Manton noticed 10-17 quadrivalents in 92 cells out of 101 examined and in one cell each 19 and 21 quadrivalents were present. In addition, she observed in 2/3 of the cells, one to two trivalents and up to a maximum of five in only two cells. It may be recalled that *O. regalis* has n=22 and *A. capillus-veneris* n=30. The percentage of quadrivalents in *O. regalis* is 66 while in the present case it is 40 and trivalents are absent. The lower frequency of quadrivalent formation in *A. capillus-veneris* is probably due to the smaller size of chromosomes in this species as compared to *O. regalis*. This may also account for the almost normal anaphases in the present species. This is in line with the view advanced by Darlington (1937), Darlington and Mather (1949) and Kostoff (1940).

One of the striking observations is the presence of certain percentage of spores with bilateral symmetry amongst the largest number with tetrahedral symmetry. Colchicine seems to have somehow disturbed the normal course of cytokinesis in certain spore mother cells.

The occurrence of perfectly normal disjunction resulting in normal spores by the autotetraploid in *A. capillus-veneris* is another important feature. The viable nature of these spores has been confirmed by actual germination and the cultures are in progress and being studied by the authors. The reason for the normal meiosis and spores may lie in the fact that the present species is diploid throughout its range, and that in the genus there already exist potentialities for polyploidy up to the hexaploid level (apogamous). Furthermore, the heterozygous nature of this horticultural fern is an additional point in favour. These are the two important pre-requisites for raising autoploids. The present experiment is encouraging and the technique is being employed in various other ferns of cytotaxonomic interest.

Summary

The previous work concerning induction of autopolyploids in ferns has been reviewed. So far, colchicine has never been used successfully in raising autoploids in ferns, and even by apospory, Manton alone has been able to study meiosis in fertile autoploid fronds of *O. regalis*.

The authors have found colchicine as a much quicker and surer method for raising autoploids in ferns. Immersion of rhizomes in aqueous colchicine of 0·5 per cent concentration for nearly 48 hours has been found very successful.

Morphological and cytological comparisons between the diploid and autotetraploid of *A. capillus-veneris* have been made. At meiosis, only quadrivalents and bivalents are present which disjunct normally, resulting in the normal output of 64 spores in a sporangium. These spores are now in cultures and being studied by the authors. Another interesting feature of the present study is the presence of three to five per cent of spores with bilateral symmetry which perhaps is due to colchicine action itself. The pairing analysis in the present autotetraploid has been compared with the autotetraploid *O. regalis* investigated by Manton (1950) with almost identical results. However, there is a less frequency of quadrivalents and complete absence of trivalents in the present autotetraploid, which may be due to the small size of the chromosomes.

These autotetraploid series would help in biosystematics, in analysing genome relationships, genetic studies with the help of trisomics and raising horticultural types.

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INHERITANCE OF PALE-GREEN COLOUR IN COTTON*

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SEVERAL types of chlorophyll deficiency have been discovered and their breeding behaviour studied extensively in *Gossypium hirsutum* and *G. barbadense*, both in the seedling and adult stages. But in *G. herbaceum* these characters have been studied to a very limited extent, and that too only in the seedling stage.

A few chlorophyll abnormalities have been reported by Patel *et al.* (1946) in the Indian *herbaceum* cottons, but not investigated genetically and physiologically as in the above species.

A pale-green variant found in Broach cottons was the first case of chlorophyll deficiency so far known in the Indian *herbaceum* cottons. Hence, it was considered worthwhile to study its behaviour genetically and physiologically and also study its relation to the economic characters, such as ginning outturn, staple length and seed weight.

The chlorophyll deficiencies in cotton have been studied by Balls (1908), Chi (1939), Harland (1932), Horlacher and Killough (1931) and Killough and Horlacher (1933).

Stroman and Mahoney (1925) recovered two chlorophyll deficient types in the F_2 of the crosses of Upland and Egyptian cottons (*G. hirsutum* × *G. barbadense*). The inheritance of the first type *yellow* seedling was governed by duplicate factors. The second type of chlorophyll deficiency was a pattern seedling character in which irregular areas devoid of chlorophyll on the cotyledon leaves were surrounded by normal green pigment. These pattern characters were inherited as recessive, in some cases controlled by two and in others by three pairs of genes.

Ware (1927), in a cross between the red leaf and the green leaf Egyptian cottons, found F_1 plants with dilute-red leaves and the F_2 generation segregated into 1 red : 2 dilute-red : 1 green.

Texas workers (1928) found a virescent yellow mutant in Mebane, a variety of *hirsutum* cotton. The mutant virescent yellow behaved as a recessive and the factor pair responsible was designated V-v. They further observed that plants with variegated leaves were frequently found in both *barbadense* and *hirsutum*, and that the inheritance of this character was cytoplasmic.

Material and methods

The experimental material consisted of two pure types of Broach cotton, viz., B.C. 1-2 and Pale Green Leaf.

B.C. 1-2: It is a standard cotton named Vijay. It is a wilt-resistant, high-ginning and high-spinning type with a normal green colour of the foliage and bolls.

Pale Green Leaf (P.G.L.): This mutant was isolated from a single plant progeny trial of the strain B.C. 7-29 in the year 1939-40 at the Cotton Breeding Station, Broach, Gujarat State. This mutant has pale-green leaves from the seedling stage which persist throughout the life of the plant. The colour of the boll is also pale-green. The morphological and economical characters of the two types used in this study are summarized in Table I.

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*Part of thesis submitted to Gujarat University by the senior author in partial fulfilment of the requirements for the Degree of Master of Science.

The authors' thanks are due to Dr. M. D. Patel, formerly Director, Institute of Agriculture, Anand, for facilities and encouragement during this work.

TABLE I. MORPHOLOGICAL AND ECONOMIC CHARACTERS OF THE TWO PARENTAL TYPES

Material	Foliage colour	Boll colour	Yield of seed cotton in lb. per acre	Ginning percentage	Staple length (mm.)	Spinning value (H.S.W.C.)
*B.C. 1-2 (Vijay) ..	Normal green	Normal green	514	41·2	0·86	38
*P.G.L.	Pale-green	Pale-green	521	42·4	0·84	36

*Performance at the Broach Cotton Breeding Station, Gujarat State.

Except the colour of foliage and boll and that P.G.L. matures about a fortnight earlier, these are identical in most plant characters.

To study the behaviour of the pale-green leaf mutant with the normal green B.C. 1-2, reciprocal crosses were made in 1949. The material was advanced to the F_2 generation and studied for two seasons, viz., 1951 and 1952. Besides the F_2 generation, all possible combinations of the first back-crosses were made in order to have confirmation of the F_2 generation data. These first back-crosses were studied in the 1952 season along with the F_1, F_2 and the parental material.

The reciprocal crosses were made not only to verify the mode of inheritance of the pale-green character but also to detect if there was any difference in the quantity of chlorophyll, considering the nature and place of occurrence of progenitors of this character, viz., the plastids which are mostly confined to ovules. This was studied both quantitatively and qualitatively by a chemical analysis of the chlorophyll content.

Foliage, boll colour and their relation to economic characters, viz., ginning percentage, staple length and seed weight were studied.

Experimental results

Part A: The data on the study of foliage and boll colour in the F_1 and F_2 and back-cross generations of the crosses of P.G.L. with B.C. 1-2 are presented in Tables II to V. They are grouped as follows:

Group I. F_1, F_2 and back-crosses of P.G.L. used as a female with B.C. 1-2 used as a male. Tables II and III.

Group II. F_1, F_2 and back-crosses of B.C. 1-2 used as a female with P.G.L. as a male. Tables IV and V.

Part B: The results of the chemical analysis of the chlorophyll content of the leaves of the parents, F_1, F_2 and back-cross generations are given in Table VI.

Part C: The data on the relation of foliage colours of the hybrid generations with ginning percentage, staple length and seed weight are summarized.

PART A

Study of foliage and boll colour

Group I. Pale-green parent used as female.

F_1 Hybrid. P.G.L. × B.C. 1-2—colour of foliage and bolls of the F_1 plants was dilute-green.

An examination of the data presented in Table II shows that the F_2 population segregated into three different classes, viz., normal green, dilute-green and pale-green, and the X^2 test showed excellent fit of the data for a monogenic inheritance (1 : 2 : 1).

The data of back-crosses of F_1 with the maternal pale-green parent and the paternal normal green parent are given in Table III.

TABLE II. SEGREGATION OF LEAF AND BOLL COLOUR IN F_2 GENERATION

Cross	Year of study		No. of plants in F_2			Total	X^2	P
			Normal green	Dilute green	Pale-green			
(P.G.L. × B.C. 1-2) F_2 ..	1951	Observed	51·00	98·00	41·00	190	1·22	0·50 to 0·70
		Expected 1:2:1	47·50	95·00	47·50	190		
	1952	Observed	237·00	460·00	241·00	938	0·40	0·80 to 0·90
		Expected 1:2:1	234·50	469·00	234·50	938		
	Both the years	Observed	288·00	558·00	282·00	1,128	0·19	0·90 to 0·95
		Expected 1:2:1	282·00	564·00	282·00	1,128		

TABLE III. SEGREGATION OF LEAF AND BOLL COLOUR IN BACK-CROSS GENERATIONS

Sl. No.	Back-cross		No. of plants in back-cross F_1			Total	X^2	P
			Normal green	Dilute green	Pale-green			
A	{(P.G.L. × B.C. 1-2) F_1 × P.G.L. }F_1 ..	Observed	..	172·00	180·00	352	0·18	0·50 to 0·70
		Expected 1 : 1	..	176·00	176·00	352		
B	{P.G.L. × (P.G.L. × B.C. 1-2) F_1 } F_1	Observed	..	163·00	157·00	320	0·112	0·70 to 0·80
		Expected 1 : 1	..	160·00	160·00	320		
C	{(P.G.L. × B.C. 1-2) F_1 × B.C. 1-2 }F_1	Observed	154·00	145·00	..	299	0·26	0·50 to 0·70
		Expected 1 : 1	149·50	149·50	..	299		
D	{B.C. 1-2 × (P.G.L. × B.C. 1-2) F_1 }F_1	Observed	150·00	162·00	..	312	0·46	0·50
		Expected 1 : 1	156·00	156·00	..	312		

The results presented in Table III show that the back-cross populations A and B consisted of two types of plants, namely, dilute-green and pale-green, and back-cross populations C and D consisted of normal green and dilute green plants.

There is practically no deviation of the observed values from those of the expected. The probability based on the X^2 values show a good fit for a 1 : 1 ratio, thus confirming the F_2 results.

Group II. Normal green parent used as female.

F_1 Hybrid. B.C. 1-2 × P.G.L.—All the F_1 plants in this cross were dilute green as in the cross of P.G.L. with B.C. 1-2, but upon minute examination they appeared more greenish in comparison to those of P.G.L. × B.C. 1-2.

The data given in Table IV show that the F_2 populations were of three groups as

observed in the previous cross of P.G.L. with B.C. 1-2.

The observed values are not very much different from those of the expected, except in 1951. The X^2 values, both for the data of 1952 and for the combined data of 1951 and 1952 show a good fit for a monogenic F_2 ratio of 1 : 2 : 1.

The data of back-crosses of F_1 with the paternal pale-green parent and the maternal normal green parent are summarized in Table V.

The results presented reveal that the back-cross populations E and F were of two groups, viz., dilute-green and pale-green, and back-cross populations G and H were of normal green and dilute green plants.

The observed values are very near to the expected ones and the X^2 values show a good agreement for the 1 : 1 ratio, thus confirming the F_2 results.

It becomes evident from the above results that the relation of these two chlorophyll

TABLE IV. SEGREGATION OF FOLIAGE AND BOLL COLOUR IN THE F_2 GENERATION

Cross	Year of study		No. of plants in F_2 Normal green	Dilute green	Pale-green	Total	X^2	P
(B.C. 1-2 × P.G.L.) F_2 ..	1951	Observed	50·00	109·00	39·00	198	3·21	0·20 to 0·30
		Expected 1:2:1	49·50	99·00	49·50	198		
	1952	Observed	188·00	370·00	174·00	732	0·61	0·70 to 0·80
		Expected 1:2:1	183·00	366·00	183·00	732		
	Both years	Observed	238·00	479·00	213·00	930	0·71	0·70
		Expected 1:2:1	232·50	465·00	232·50	930		

TABLE V. SEGREGATION OF FOLIAGE AND BOLL COLOUR IN THE BACK-CROSS GENERATION

Sl. No.	Back-crosses		No of plants in back-cross F_1 Normal green	Dilute green	Pale-green	Total	X^2	P
E	{(B.C. 1-2 × P.G.L.) F_1 × P.G.L. }F_1	Observed	..	182·00	164·00	346	0·92	0·30 to 0·50
		Expected 1 : 1	..	173·00	173·00	346		
F	{P.G.L. × (B.C. 1-2 × P.G.L.) F_1 }F_1	Observed	..	178·00	185·00	363	0·134	0·70 to 0·80
		Expected 1 : 1	..	181·50	181·50	363		
G	{(B.C. 1-2 × P.G.L.) F_1 × B.C. 1-2 }F_1	Observed	85·00	72·00	..	157	1·06	0·30 to 0·50
		Expected 1 : 1	78·50	78·50	..	157		
H	{B.C. 1-2 × (B.C. 1-2 × P.G.L.) F_1 }F_1	Observed	41·00	40·00	..	81	0·012	0·90 to 0·95
		Expected 1 : 1	40·50	40–50	..	81		

traits is allelic and monogenic with incomplete dominance of normal green over the pale-green, as evidenced by the dilute green trait of the F_1s and F_2 populations segregating in the proportion of 1 normal green : 2 dilute green : 1 pale-green.

The above results were confirmed by the back-cross segregation in the proportion of 1 : 1 ratio of the dilute green and the parental types either the normal green or pale-green, depending upon the back-cross parent involved.

PART B

Inheritance of chlorophyll content

This part of the study was carried out in order to verify the validity of the visual classification of the three types of plants, namely, normal green, dilute green and pale-green in the segregating population of the crosses studied under Part A. The study involved an actual quantitative determination of the chlorophyll contents of the parents, F_1, F_2 and back-cross plants by chemical methods. The chlorophyll determinations were carried out for both the years under study.

The chemical procedure for determination of the chlorophyll content in the plant tissue used in this study was that described by Schertz (1928a, b).

The chlorophyll content in per cent for (i) parents, (ii) F_1's and (iii) F_2 of the cross P.G.L. × B.C. 1-2 and B.C. 1-2 × P.G.L. along with the back-cross generations were determined during the course of the investigation. The data on the chlorophyll content in per cent are given in Table VI.

An examination of the data presented in Table VI reveals that the chlorophyll content of the normal green parent (B.C. 1-2) is nearly twice that of the pale-green parent (P.G.L.) at the commencement of the flowering.

TABLE VI. CHLOROPHYLL CONTENT IN PER CENT FOR PARENTS, F_1'S AND F_2'S AND BACK-CROSS GENERATIONS OF THE PALE-GREEN LEAF WITH NORMAL GREEN LEAF

Sl. No.	Name of the parent or the cross	Stage of the crop: At flowering (1951)	One month after flowering (1951)	At flowering (1952)
1.	P.G.L.	0·175	0·106	0·118
2.	B.C. 1-2 ..	0·276	0·168	0·246
3.	(P.G.L. × B.C. 1-2)F_1	0·193	0·140	0·144
4.	(B.C. 1-2 × P.G.L.) F_1	0·206	0·165	0·165
5.	{(P.G.L. × B.C. 1-2) F_1 × P.G.L. }F_1			
	Dilute	..	..	0·183
	Pale	..	..	0·113
6.	{P.G.L. × (P.G.L. × B.C. 1-2) F_1 }F_1			
	Dilute	..	..	0·149
	Pale	..	..	0·106
7.	{(P.G.L. × B.C. 1-2) F_1 B.C. 1-2 }F_1			
	Normal	..	..	0·215
	Dilute	..	..	0·165
8.	{B.C. 1-2 × (P.G.L. × B.C. 1-2) F_1 }F_1			
	Normal	..	..	0·236
	Dilute	..	..	0·179
9.	{(B.C. 1-2 × P.G.L.) F_1 × P.G.L. }F_1			
	Dilute	..	..	0·168
	Pale	..	..	0·117
10.	{P.G.L. × (B.C. 1-2 × P.G.L.) F_1 }F_1			
	Dilute	..	..	0·146
	Pale	..	..	0·107

TABLE VI—(*Concld.*)

Sl. No.	Name of the parent or the cross	Stage of the crop		
		At flowering (1951)	One month after flowering (1951)	At flowering (1952)
11.	{(B.C. 1-2 × P.G.L.) F_1 × B.C. 1-2 }F_1			
	Normal ..	..	..	0·166
	Dilute ..	..	..	0·145
12.	{B.C. 1-2 × (B.C. 1-2 × P.G.L.) F_1 }F_1			
	Normal ..	..	..	0·211
	Dilute ..	..	..	0·161
13.	(P.G.L. × B.C. 1-2)F_2			
	Normal ..	0·260	0·150	0·186
	Dilute ..	0·207	0·125	0·138
	Pale ..	0·175	0·118	0·123
14.	(B.C. 1-2 × P.G.L.)F_2			
	Normal ..	0·255	0·143	0·166
	Dilute ..	0·233	0·120	0·123
	Pale ..	0·154	0·107	0·105

The chlorophyll content of the two reciprocal hybrids (three and four) is intermediate between the parents. But there is difference in the chlorophyll content of the two F_1 hybrids, in that the hybrid with P.G.L. as female contained less chlorophyll than that with normal green (B.C. 1-2).

In the case of back-cross results also there are differences in the chlorophyll content of the dilute green and pale-green classes of the two back-crosses with the P.G.L. parent, viz., P.G.L. as male and female parent.

Similar differences in the chlorophyll content exist between the dilute green types resulting from reciprocal back-crosses, involving the normal green parent. That is, the chlorophyll content of the dilute green class is more when B.C. 1-2 is used as a female parent than when B.C. 1-2 is used as a male parent. The same relation is also evident from the data with regard to the normal green class.

These quantitative chlorophyll discrepancies in the progeny of the F_1 and back-crosses could be reasonably explained on the presence of the green plastids in the cytoplasm of the ovules of the female parent which are absent in the male gametes. That is why two dilute green classes of the two reciprocal F_1's and the population of the reciprocal back-crosses differ in the quantity of chlorophyll in the leaf.

The data of F_2 generations on the chlorophyll content of the three types, namely, normal green, dilute-green and pale-green, also show a fairly good relation with their visual classification and their actual chlorophyll contents. The chlorophyll content decreased with the decrease in the intensity of the greenness in these three classes in order of normal green, dilute green and pale-green.

PART C

Relation of chlorophyll character and economic characters

This study was conducted to ascertain whether there was any influence of chlorophyll traits on the ginning percentage, staple length and development of the seed.

No significant differences in the ginning percentage, staple length and seed weight were observed between normal green and dilute-green plants, dilute-green and pale-green plants and normal green and pale-green plants.

Summary

Inheritance studies of chlorophyll deficiency in Broach cotton were made in the crosses of pale-green leaf (P.G.L.) with normal green leaf (B.C. 1-2) and its reciprocal.

The F_1 hybrid from a cross of P.G.L. and B.C. 1-2 and its reciprocal is dilute green, showing incomplete dominance of normal green over pale-green, but there is a difference between the two F_1 hybrids with regard to the intensity of greenness. When the pale-green leaf is used as a female parent, the chlorophyll of the F_1 hybrid is less than when the normal green (B.C. 1-2) is used as a female parent, as corroborated by an actual chemical analysis of the chlorophyll.

The results of two F_2 and back-cross generations indicate that the pale-green chlorophyll deficiency is inherited in a monogenic fashion. The F_2 population segregated into 1 normal green : 2 dilute-green : 1 pale-green.

The F_1 when back-crossed to the pale-green parent, as a female and male parent, the ratio in both populations is 1 dilute-green : 1 pale-green, but two dilute-greens show a visual difference with regard to the green colour.

When the F_1 is back-crossed with the normal green parent directly and reciprocally, the ratio is 1 normal green : 1 dilute-green. However, there is difference between the two normal green classes with regard to the intensity of greenness. When normal green is used as a female parent, the chlorophyll content is more than when used as a male parent.

These visual field differences in between two normal green classes, in between two dilute green classes and also in between two pale-green classes in these crosses are also supported by the chemical analysis of their chlorophyll contents.

The inheritance of two chlorophyll traits, namely, normal green and pale-green, is governed by a single pair of genes and is considerably influenced by the maternal parent. This can be reasonably expected, since the primordia of chlorophyll occur in the cytoplasm.

There was no evidence of any influence of the chlorophyll types on any of the three characters, viz., ginning percentage, staple length and seed weight.

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